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Section C

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STUDIES IN TREE PHYSIOLOGY

II. SEASONAL CHANGES IN THE FOOD RESERVES OF FIELD BIRCH (*BETULA POPULIFOLIA* MARSH.)[1]

45988

19 OCT 1943

BY R. DARNLEY GIBBS[2]

Abstract

During the period January 1936 to July 1938, the upper parts of *Betula populifolia* Marsh. were analysed for water, reducing sugars, sucrose, total soluble sugars, ether-soluble material, polysaccharide hydrolysable by taka-diastase, and residual polysaccharides extracted by dilute acid. From 38 to 63% of the taka-diastase hydrolysate from the wood is non-fermentable, presumably pentose. The residual acid extract is from 69 to 100% non-fermentable. Hexosan is low in winter, rises to a maximum just before leaf-opening, decreases sharply during spring growth and accumulates in summer. Ether-soluble material is not stored in winter. Sucrose and to a lesser extent reducing sugars are high in winter. There is a significant correlation between reducing sugars and water content of the twigs. Food reserves of the cortex behave much as do those of wood.

Introduction

In this paper, the second of a projected series on tree physiology, are recorded the results of analyses extending over two and a half years (from January 1936 to July 1938) of the upper parts of field birch. The trees used were young specimens, 15 to 20 ft. high and with a D.B.H. of 2 to 4 in., growing in a field (which they had invaded) at Ste. Anne de Bellevue, about 20 miles from Montreal. The water contents of these and other trees were reported in the first paper of this series (8).

The seasonal changes in deciduous trees in Eastern Canada are very marked. There is almost continuous frost from November until April and this is reflected in the "dormant" condition of the trees during this period. Leaves expand in early May and are dropped in October. Food manufactured in the leafy season is stored within the tree during the winter, to be used with the onset of growth in late April. This storage of food in winter is only one aspect of the physiology of the tree, for correlated with this is an increase in winter hardiness which is of great interest and practical importance. Stored foods, of course, are not alone responsible for this hardiness, but they are a part of the mechanism and must be included in any complete picture.

Schultz (17), in 1888, found that starch disappears from evergreen leaves in winter, to be replaced by tannin-like materials. Suroz (20), a little later, believed starch to be replaced by oil while Fischer (7), in the same year,

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Contribution from the Department of Botany, McGill University, Montreal, Que., with financial assistance from the National Research Council of Canada.

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published an extensive paper in which he classed trees as starch- and fat-trees, the former retaining much starch in winter, the latter replacing starch by fat. Fischer considered that most of the trees with hard wood belong to the first class, while those with softer wood, and the conifers, belong to the second. His conclusions have been criticized more recently by Preston and Phillips (16). Lidforss (12, 13) first began to correlate seasonal, food-reserve changes in evergreens with resistance to winter cold.

Most of the work that has been done in this field (and it is very extensive) has been only roughly quantitative, depending in great measure upon staining of microscopic sections for starch, hemicelluloses, and fats. This is true even of such recent work as that of Ishibe (10) who stained with Sudan III in a mixture of propyl alcohol and glycerine for "fats". It is very doubtful, as Doyle and Clinch pointed out some years ago (6), if the materials staining with osmic acid, Sudan III, and other so-called "fat" stains are really fats. Doyle has again drawn attention to this in a recent note (5).

There are several quantitative methods available for starch, though the accuracy of some of them may be questioned. It is not intended to deal with these in detail. The investigation of hemicelluloses is not an easy matter and it is doubtful if an exact characterization of hemicellulose fractions can yet be made.

Materials and Methods

In the present work the upper parts, consisting of one-, two-, and three-year-old twigs, of field birch were employed. Except in some of the later analyses the wood alone was used. In these last, however, the material was separated into wood and "cortex" (all outside the wood) and both were analysed. Material was collected in each case from three trees, samples being taken for determination of water at the same time (8). These were dried at 105° C. in an electric oven, which has been shown to give accurate results with wood of birch (9). The twigs were brought quickly into the laboratory, wood and cortex separated as rapidly as possible, and the wood roughly ground in a pencil sharpener. The ground material was then sampled into four lots with a Jones sampler; the lots, each of about 10 gm., were weighed, and two were dried at 105° C. in an electric oven, while the others were used at once for analysis. This second water determination permits of the reference of all figures to a dry weight basis.

The fresh samples were thrown into boiling alcohol to which a pinch of precipitated calcium carbonate had been added. The calcium carbonate was supposed to neutralize any acids present and so to prevent hydrolysis of any sugars, but in a paper which was brought to our attention after our analyses were started Denny (2) questioned the use of carbonate in this connection. However, since carbonate was added in the early analyses its use was continued throughout the series. The alcohol employed was of sufficient strength to give, with the water in the tissue, a final strength of about 80%. It was poured off and the extraction was repeated several times.

The samples were then transferred to a small Pyrex glass ball-mill and ground in 95% alcohol for 6 to 7 hr. The alcohol was filtered off under suction, the cake broken up and extracted exhaustively with ether in an all-glass Soxhlet apparatus having "standard-taper" joints.

The alcoholic extracts were combined and the alcohol evaporated off under reduced pressure. The residual liquid (mostly water) contained the soluble sugars and a scum of fat, pigments, etc., which had been extracted by the alcohol. The mixture was therefore thoroughly ether-extracted by repeated shaking in a separatory funnel.[1] The ether extracts so obtained were added to the Soxhlet extract, and the whole evaporated down and dried at 100° C. as *ether soluble material*. It consisted, of course, of much more than true fat, but was not further differentiated.

The aqueous residue from the separatory-funnel extractions contained the soluble sugars. It was cleared by neutral lead acetate followed by potassium oxalate[2] and aliquots were analysed for *reducing sugars* by the method of Shaffer and Somogyi (18).

Further aliquots were quantitatively inverted by "convertit", a commercial invertase preparation[3], and analysed for *total soluble sugars*. By difference, values for *sucrose* were obtained. As will be seen later, field birch contains considerable quantities of pentosans, and it was thought that some pentose might be contained in these soluble sugar fractions. The amount, however, proved to be quite low, for the solutions gave only weak reactions for pentoses and were almost quantitatively fermentable by yeast.

The cake of ground material left after the Soxhlet extraction was dispersed in water and heated to drive off the ether and to swell and disintegrate the starch grains in the tissue fragments (3). The mixture was then buffered to approximately pH 4.5 with acetic acid plus sodium acetate and treated with special undiluted taka-diastase[4] in an incubator at 37°C. for two days, a little toluol being added as an antiseptic. A second taka-diastase treatment for two days followed and the extracts were combined, boiled, and filtered. The filtrate was almost colourless but somewhat cloudy. The sugar in this solution was estimated as above, and at first assumed to be derived entirely from "*starch*"; but it was found that strong pentose reactions were given, and careful fermentations showed that a considerable quantity—up to 65%—of this material was not fermentable (see also (3, 4) in this connection). Fermentation was therefore adopted as a standard procedure, and the reducing substances were differentiated into "hexose" and "pentose". Pentoses have not the same reducing power as hexoses with the carbonate-citrate reagent employed, but a few analyses of known pentose solutions show that the difference is comparatively small. The ball-mill grinding mentioned above is necessary only for the taka-diastase digestions. We therefore attempted

[1] *A continuous extraction apparatus was used for a while but found unsatisfactory.*

[2] *During the period October 1936 to March 1937, duplicate samples were cleared with "Norit" charcoal and analysed for sugars. The values obtained were sometimes considerably lower than those from lead-cleared extracts.*

[3] *Eimer and Amend, N.Y.*

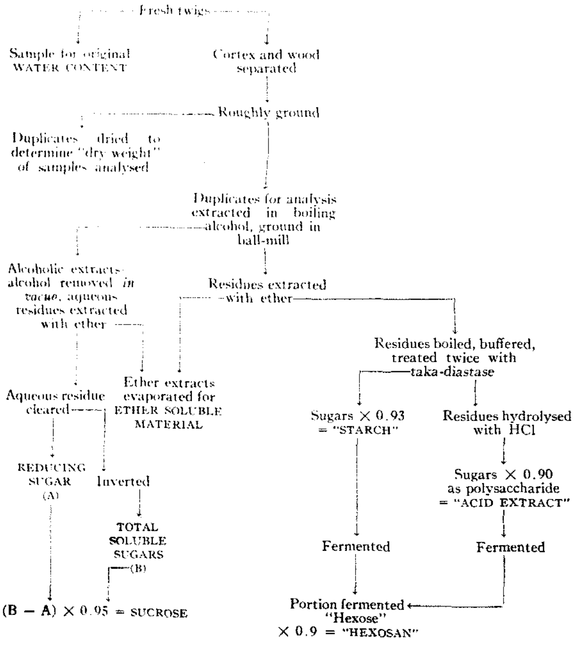
[4] *Takamine Laboratory, Inc., Clifton, N.J.*

to combine the grinding and digestion by delaying the former until after ether extraction, and then incubated with taka-diastase while grinding. This, however, was found to give very low results and was abandoned in favour of the procedure outlined above.

The *residue from taka-diastase digestion* was now treated at 100° C. for one hour with 2% hydrochloric acid. This was designed to check the completeness of extraction with taka-diastase and was found by fermentation to *yield a considerable amount of pentose but very little (or sometimes no) hexose*. We have, therefore, combined the values for "hexose" obtained by taka-diastase and acid extraction as "*Hexosan*" (chiefly starch?). The whole scheme is summarized in the flowsheet that follows.

FLOWSHEET

SCHEME OF ANALYSIS FOR TWIGS OF FIELD BIRCH



Results

Sucrose shows a regular variation during the year, rising to a maximum in December and remaining constantly high until March (Fig. 1). The maximum in the winter of 1936–7 was 2.9%, in 1935–6 it was probably about the same (2.9% in February 1936), while in the corresponding period for 1937–8 it was distinctly higher at 3.8%. A rapid decrease occurs in March-April and this decrease precedes the uptake of water, a quite interesting point. The lowest concentrations are reached in May, when leaves are expanding and growth is very rapid. The minima for 1936, 1937, and 1938 respectively were 0.5%, 0.7%, and 1.0%. The sucrose content increases slowly during the summer, until in late October it may be from 0.8% (1936) to 1.2% (1937). There is then the rapid increase to the high values noted for the winter.

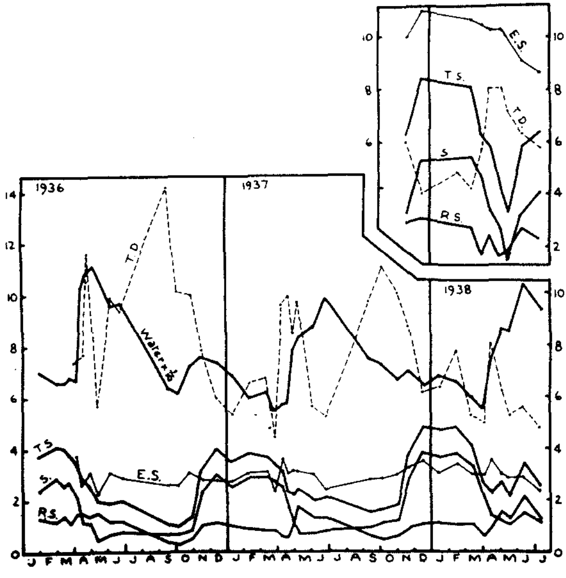


FIG. 1. *Seasonal changes in food reserves of field birch; wood below, cortex above.* R.S. = *reducing sugars;* S = *sucrose;* T.S. = *total soluble sugars;* E.S. = *ether-soluble material;* T.D. = *sugar obtained by taka-diastase hydrolysis calculated as "starch". The fluctuations in water content of wood are also included.*

Reducing sugars are never present in such large amounts as is sucrose nor are their fluctuations quite as regular. Values are relatively high in the winter, with minima in October and in late March or early April. The behaviour in spring seems to vary greatly from year to year. In April and May of 1936, concentrations were not markedly higher than during the winter, but in May, 1937 and in May and June of 1938 sharp increases occurred. These started from lower levels than in 1936 and reached higher maxima (1.1 to 1.5, 0.6 to 1.7, 0.6 to 2.1 in the respective years). These high spring concentrations coincided with low values for sucrose and might be due to inversion of the latter. Unlike sucrose, which increased slowly during the two summers studied, the reducing sugars decreased to very low levels in October (0.3% in 1936, 0.5% in 1937). An interesting point is the statistically significant correlation between water content and amount of reducing sugars based on dry weight (correlation coefficient = 0.66 ± 0.091). This does not mean a constant concentration of sugar in the water present, however, for actually there is a variation on this basis of from 0.5 to 2.2% in the period studied and the highest concentrations occur in winter (when water is low) and at leaf-opening (when water is high).

The curve for total soluble sugars shows a high value in winter and a low one in summer, with a minimum in September or October.

The behaviour of soluble sugars in the cortex is very similar to that in the wood (Fig. 1). During the winter sucrose is present in amounts much above that of the reducing sugars, but decreases sharply in spring to a value below them.

The present investigation tells little about the distribution of the sugars in the tissues studied, but we may hazard a guess that in the cortex most of the sugar is in the living cells while in the wood much of it is in the extra-cellular water. This is based upon a comparison of our figures with those for cortical cells of certain species given by Levitt and Scarth (11). These authors found, in their studies on frost-hardening, that the osmotic pressures of living cortical cells of *Caragana arborescens*, *Liriodendron tulipifera*, *Catalpa hybrida*, *Hydrangea paniculata*, and *Picea pungens* follow the curve given in Fig. 2 (we have averaged their results for these five plants). If we assume that living cells of the wood of field birch behave similarly this would account for only a very small amount of sugar (since the wood contains relatively few living cells). Actually we find that there is enough sugar in birch wood to give osmotic pressures, with all the water present, of as much as one-fifth of those found by Levitt and Scarth. Much of this sugar, then, is probably in the extra-cellular water. This is, perhaps, supported by the observation of the correlation between water content and reducing sugars already noted.

In cortex, on the other hand, living cells occupy a much higher proportion of the tissues. Unfortunately we have no accurate figures for water content of cortex so no direct comparisons of concentration can be made, but water contents of the samples as prepared for analysis suggest that changes in water content of fresh material were such that we could construct a curve for osmotic

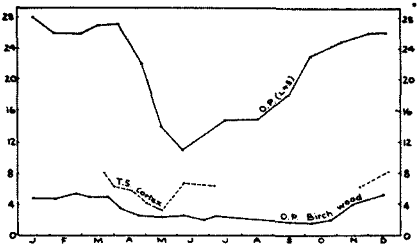


FIG. 2. *Upper curve—osmotic pressures in atmospheres of the cortical cells of hardy woody plants (Levitt and Scarth). Lower curve—osmotic pressures of soluble sugars in wood of field birch (on the assumption that the sugars are evenly distributed in the water present). Middle curve—total soluble sugars in cortex of field birch (percentage based on dry weight).*

pressure in this case which would lie slightly below that given by Levitt and Scarth but would follow essentially the same course. This would mean that much of the sugar present is in the living cells.

The ether-soluble material varies but little in amount throughout the year in the wood of field birch and is not significantly higher during the winter (Fig. 1). There is, then, no evidence here of fat storage in winter. This is not in agreement with the results of Niklewski (14) for wood of *Betula alba*, since he found decidedly more "fat" (ether extract) in late October and January than in August and February. There is some evidence of a high ether-extract from cortex of field birch in winter (Fig. 1) but our analyses for this part of the tree do not cover a sufficiently long period for any generalizations to be made. It is hoped, in later work, further to characterize the ether-extract.

Turning now to the material hydrolysed by taka-diastase—which might be listed as starch, but that it is only partially fermentable—we find in the wood great variations from year to year, but a general picture recalling that of Fischer (*loc. cit.*) for starch. Comparatively low values are found in winter. There is then a sharp increase just before leaf opening (not well marked in 1938) and an equally sharp decrease during the early stages of growth. Sugar changes seem not to be sufficient to account for the spring increase, but it must be remembered that we have taken no account of possible translocation in the present investigation. Storage of material hydrolysable by taka-diastase occurs during summer and the highest recorded values are reached in September or early October. There is then a rapid decrease which coincides with the increase in soluble sugars.

When the taka-diastase hydrolysate is fermented there is a fairly close correlation between the amount of hydrolysate and the proportion fermented (correlation coefficient = 0.54 ± 0.176)—*i.e.*, the non-fermentable ("pentose") proportion is not varying as much as the fermentable ("hexose") fraction.

An essentially similar relation is seen, as far as our figures go, in the cortex, with a low "starch" value in winter, a rapid increase in spring and a sharp decline following leaf-opening. The correlation between taka-diastase hydrolysate and proportion of hydrolysate fermentable is even closer in the cortex than it is in the wood.

The acid extract was used, as noted, to test the completeness of extraction of hexosan by taka-diastase, and it is probable that no very definite fraction is extracted here, at least under the condition of the experiments. Usually it is almost all pentose.

When the "hexose" fractions of the taka-diastase and acid hydrolysates are combined we get figures for easily-extracted "*hexosan*" (probably mostly starch), which show the type of seasonal variation which Fischer believed to be characteristic of starch in many trees (Fig. 3). The close parallel between the hexosan and taka-diastase hydrolysate curves make it possible to use the latter as a measure of "starch" content.

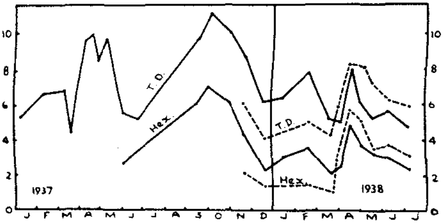


FIG. 3. *Seasonal changes in "polysaccharides" of field birch.* Hex. = "*hexosan*"; T.D. = *sugar obtained by taka-diastase hydrolysis, calculated as "starch". Solid lines for wood, dotted for cortex.*

One last point which is of considerable interest may be noted here. In trying to explain the seasonal changes in food reserves of trees, Sinnott (19) suggested that ease of access of water to storage cells might be a great factor, possibly through carriage of enzymes. A consideration of our curves for water content and starch, however, shows that the formation of starch in spring is quite independent of changes in water content. Thus, in 1936 a marked increase in starch occurred some time after the spring increase in water content, in 1937 it occurred well before any seasonal change, and in 1938 practically coincided with it.

Acknowledgments

The author wishes to acknowledge the kindness of Mr. Cleveland Morgan in permitting the use of trees from his land at Ste. Anne de Bellevue. Dr. F. E. Denny, of the Boyce Thompson Institute, provided valuable information on methods for the estimation of starch, while Dr. S. C. Reed assisted with calculations of significance and correlation. Our thanks are tendered to them all.

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NEW SPECIES OF GYMNOSPORANGIUM[1]

By Ivan H. Crowell[2]

Abstract

Gymnosporangium minus n. sp. occurs on *Cupressus sempervirens* L. near Athens, Greece, and is the only species on a non-juniperus telial host in Europe. *G. guatemalianum* n. sp. was found on *Amelanchier nervosa* (Dcne.) Standl. and *G. meridissimum* n. sp. on *Cupressus benthami* Endl. in Guatemala; they are the most southerly species of the genus and may be genetically related.

1. *Gymnosporangium minus* Crowell, sp. nov., III (Fig. I).

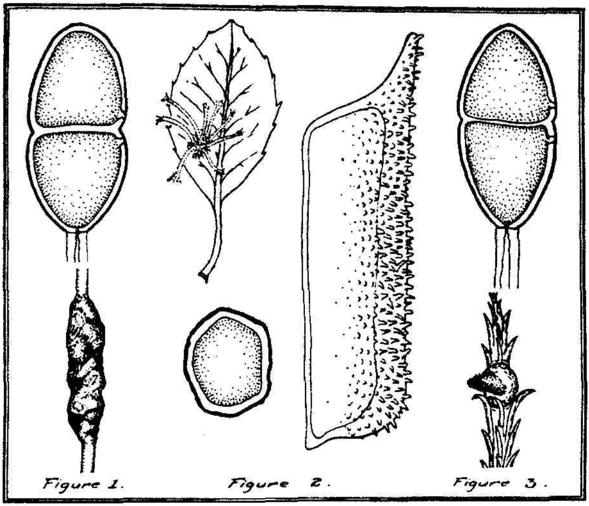
Pycnidia et aecidia ignota. Uredinia desunt. Soris teleutosporiferis caulicolis, pulvinatis, flavobrunneis vel castaneobrunneis; teleutosporis ellipsoideis, medio 1-septatis, rarius non-septatis, leviter constrictis mediis, $17.5 - 24.5\mu \times 45.5 - 59.5\mu$ plus minusve $22.3\mu \times 50.4\mu$, quaque cellula poris germinationis 2 prope septum, pedicello brevi, hyalino.

Habitat: soris teleutosporiferis in *Cupressus sempervirens L.* "Collected near Athens, on the road to Aleusis, Greece," April 3, 1906. A. S. Pease No. 8924½. Type specimen deposited in the Farlow Herbarium of Harvard University.

Although several species of *Gymnosporangium* attack a group of closely related telial hosts, many are confined to a single species; one only, namely, *G. clavipes*, parasitizes hosts in two sections of the genus *Juniperus*, but no species is known to attack hosts in more than a single genus of telial hosts. Furthermore, all these rusts on non-juniperus telial hosts, as *Cupressus*, are of local distribution. *G. minus* is the only species of *Gymnosporangium* on a non-juniperus telial host recorded from Europe.

In Oudeman's Enumeratio Systematica Fungorum 1: 663, 1919, *G. sabinae* (Dicks.) Wint. is reported on *Cupressus sempervirens*, but additional data are not given. In view of the host specificity in the genus *Gymnosporangium* and the lack of cultural data to prove otherwise, it appears that *G. minus* is a valid species. It is assumed that an error was made in the determination of *G. sabinae* as occurring on *Cupressus sempervirens*. Kern (1911) in his "A Biologic and Taxonomic Study of the Genus Gymnosporangium" states that teliospores of *G. sabinae* measure $19–30\mu \times 37–48\mu$, and are usually narrowed at both ends. Teliospores of *G. minus* are by my own comparisons narrower, considerably longer and more rotund terminally than those of *G. sabinae*.

[1] *Manuscript received August 29, 1930.*
Contribution from the Faculty of Agriculture of McGill University, Macdonald College, Que. Macdonald College Journal Series No. 132.
[2] *Lecturer in the Department of Plant Pathology, Macdonald College, Que.*



FIGS. 1–3. FIG. 1. *Gymnosporangium minus Crowell, n. sp.* FIG. 2. *Gymnosporangium guatemalianum Crowell, n. sp.* FIG. 3. *Gymnosporangium meridissimum Crowell, n. sp.*

2. *Gymnosporangium guatemalianum* Crowell sp. nov., O-I (Fig. 2).

Spermogonia foliicolae, subepidermalia. Aecidia hypophylla, roestelioidea, tumoribus tumidis insidentia 5–8 mm. alta, 0.25–0.5 mm. lata, cellulae peridii elongatae, hyalinae, pariete interiore spinulosae, marginibus gradatim plus breviter spinulosae, 24–32μ × 80–120μ plus minusve 27.6μ × 106.8μ; aecidiosporae globosae, pallide brunneae, verruculosae, 20–24μ × 24–30μ, plus minusve 21.4μ × 26.4μ.

Habitat: in foliis *Amelanchier nervosa* (Dcne.) Standl. in Parramos, Guatemala, December 14, 1938, J. R. Johnston No. 1444. Uredinia desunt. Soris teleutosporiferis ignota.

Type specimen deposited in Arthur Herbarium, Purdue University, Lafayette, Indiana. Co-type in Herbaria de la Escuela Nacional Central de Agricultura, Chimaltenango, Guatemala, and Macdonald College Mycological Herbarium, Macdonald College, Que.

3. *Gymnosporangium meridissimum* Crowell, sp. nov., III (Fig. 3).

Pycnidia et aecidia ignota. Uredinia desunt. Soris teleutosporiferis caulicolis, pulvinatis, flavobrunneis vel castaneobrunneis; teleutosporis ellip-

soideis, medio 1-septatis, leviter constrictis mediis, 24–28μ × 48–60μ plus *minusve* 25.6μ × 51.8μ, quaque cellula poris germinationis 2 prope septum, aliquando 1 prope septum et 1 prope apex, pedicello longi, hyalino.

Habitat: soris teleutosporiferis in *Cupressus benthami* Endl. = *C. lusitanica* Mill. in Alameda, Guatemala, April 1937. J. R. Johnston. Also found in Antiqua, Guatemala.

Type specimen deposited in Arthur Herbarium, Purdue University, Lafayette, Indiana. Co-types in Herbario de la Escuela Nacional Central de Agricultura, Chimaltenango, Guatemala, and Macdonald College Mycological Herbarium, Macdonald College, Que.

Small, rotund galls with one or two telia are produced on the young twigs and bear the first crop of spores. Most of these galls die shortly afterwards, but infections occasionally persist and form characteristic, deeply-fissured, fusiform swellings on lateral branches and the main trunk. These swellings vary in size up to approximately a foot in length. This is the only species of *Gymnosporangium* on a non-juniperus telial host known to form rotund galls. *G. guatemalianum* and *G. meridissimum* are possibly different stages of the same species.

The Guatemalian stations for these species of *Gymnosporangium* are the most southerly on record for the genus. *G. clavipes* found near Mexico City, Mexico, is the only other species reported south of the United States in continental North America. The hosts for these species, as well as for the one from Europe, are new for the genus *Gymnosporangium*.

VEGETATIVE PROPAGATION OF CONIFERS

IV. EFFECTS OF CANE SUGAR, ETHYL MERCURIC PHOSPHATE, AND INDOLYLACETIC ACID IN TALC DUST ON THE ROOTING OF NORWAY SPRUCE[1]

By N. H. Grace[2]

Abstract

Norway spruce cuttings collected in November from the upper part of the tree were treated with a series of talc dusts containing indolylacetic and naphthylacetic acids, each at 0, 1000, and 5000 p.p.m., combined with cane sugar at 0 and 10%, and ethyl mercuric phosphate at 0 and 50 p.p.m., and propagated in a greenhouse. Indolylacetic acid at 1000 p.p.m. increased rooting by about 10% to 42.5%, three months after planting, but at 5000 p.p.m. reduced it significantly. Naphthylacetic acid reduced rooting at both concentrations. Indolylacetic acid increased the length of root per rooted cutting. Sugar in combination with indolylacetic acid inhibited the increase in root length caused by the hormone alone; organic mercury alone or in combination with indolylacetic acid reduced root length, but sugar and mercury in combination exerted no greater inhibition than either alone.

Mean root length was affected only by indolylacetic acid, which increased it.

Previous studies have shown that treatment of plant cuttings with a series of talc dusts containing indolylacetic acid, cane sugar, and ethyl mercuric phosphate may be advantageous (4). The present communication describes an experiment in which dormant Norway spruce cuttings, collected in November, 1938, were treated with a similar series of dust preparations.

Experimental

The experiment was of factorial design, comprising treatments with indolylacetic and naphthylacetic acids each at three levels, namely, 0, 1000, and 5000 p.p.m., in combination with cane sugar at 0 and 10%, and ethyl mercuric phosphate at 0 and 50 p.p.m.; the carrier dust was talc. The series of 20 treatment combinations of indolylacetic and naphthylacetic acids (separately) with cane sugar and organic mercury was included in each of six randomized blocks, the experimental unit being a group of 10 cuttings. There were thus six replicates, of which three were planted in sand and three in a mixture of sand and peat in equal proportions.

Branches were collected in November from the upper region of trees in a plantation of Norway spruce approximately 18 years of age and situated at the Petawawa Forest Experiment Station, Chalk River, Ontario. The bases of the branches were placed in moist peat, and they were stored outside until 21 December, 1938, when cuttings were prepared and treated.

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Part of a co-operative project of the Subcommittee on Forest Tree Breeding, Associate Committee on Forestry. N.R.C. No. 872.

[2] *Biochemist.*

Cuttings ranged in length from 2 to 3½ in. and did not have a heel. Each group of 10 contained four short and three of each of two other length classes. There were approximately four laterals to one branch terminal cutting. The experiment required a total of 1200 cuttings. Groups of 10 cuttings were treated by dipping the basal ends in the various dust preparations to a depth of about one-half inch, and excess dust was shaken off. The cuttings were planted immediately in propagation frames equipped with bottom heat cables. Both media were maintained at about 72° F., while the greenhouse temperature was around 65° F. The frames were provided with factory cotton screens, which reduced light and maintained humidity. The cuttings were removed three months after planting.

Results

Records were made of the number of cuttings rooted, the number of roots and their length were determined, and the mean root length was calculated. The analysis of variance of number and lengths of root per rooted cutting, and mean root length, refer to treatments with 0 and 1000 p.p.m. indolylacetic

TABLE I

ANALYSIS OF VARIANCE OF RESPONSE OF NORWAY SPRUCE CUTTINGS TO DUST TREATMENTS WITH 1000 P.P.M. INDOLYLACETIC ACID, 10% CANE SUGAR, AND 50 P.P.M. ETHYL MERCURIC PHOSPHATE

Treatments with (A) indolylacetic and naphthylacetic acids at 0, 1000, and 5000 p.p.m.; (B) indolylacetic acid at 0 and 1000 p.p.m.; cane sugar (0 and 10%) and organic mercury (0 and 50 p.p.m.) common to both A and B.

Source of variance	Degrees of freedom (A)	Mean square	Degrees of freedom (B)	Mean square		
	Number of cuttings rooted	Number of cuttings rooted†	Measurements on rooted cuttings	Number of roots per rooted cutting	Length of roots per rooted cutting	Mean root length
Between replicates						
Sand *vs.* peat-sand	1	3.9205	1	0.1875	0.75	17.16
Within media	4	1.0584	4	0.6238	607.57	137.01
Phytohormone treatment	4	5.4302***	1	0.0008	972.00**	239.86**
Sugar treatment	1	0.3381	1	0.1408	330.75*	49.82
Organic mercury treatment	1	0.0092	1	0.3008	374.08*	85.60
Interaction						
Phytohormone × sugar	4	0.1932	1	0.7008	850.08**	69.36
Phytohormone × organic mercury	4	0.1970	1	1.4008	270.75	5.14
Interaction						
Sugar × organic mercury	1	0.0126	1	0.2408	432.00*	53.13
Phytohormone × sugar × organic mercury	4	0.2044	1	0.3008	40.33	0.32
Error	95	0.1676	35	0.3658	79.84	24.08

† *Data transferred* $\sqrt{x + \frac{1}{2}}$ *basis for analysis of variance (1).*
* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

acid only. The poor rooting and numerous complete failures following treatments with 5000 p.p.m. indolylacetic acid and 1000 and 5000 p.p.m. naphthylacetic acid resulted in data unsuited to treatment by the analysis of variance procedure. The remaining data were subjected to analyses of variance with the results indicated in Table I. In Part A we see that hormone treatment had a highly significant effect on the number of cuttings rooted. None of the other treatments, nor the various interactions, attained significance. In Part B of the table we see there were no significant treatment effects on the number of roots per rooted cutting. However, length of root per rooted cutting was affected significantly by indolylacetic acid, cane sugar, and organic mercury, and there were two significant interactions, between indolylacetic acid and sugar, and between sugar and organic mercury. Mean root length was affected by indolylacetic acid—the only significant treatment effect on this measurement. In no instance did the difference between sand and peat-sand attain significance.

Table II shows the average effects, both with and without sugar and organic mercury, of indolylacetic and naphthylacetic acids, on the number of cuttings rooted. Treatment with 1000 p.p.m. of indolylacetic acid increased rooting significantly, while the 5000 p.p.m. level and both dosages of naphthylacetic acid significantly reduced rooting. Rooting at 5000 p.p.m. naphthylacetic acid was significantly below that following all other treatments.

TABLE II

ROOTING OF NORWAY SPRUCE CUTTINGS TREATED WITH INDOLYLACETIC AND NAPHTHYLACETIC ACIDS

Transformed data are means of 24 groups of 10 cuttings

	Concentration of chemical in talc, p.p.m.					Necessary difference, 5% level
	0	Indolylacetic acid		Naphthylacetic acid		
		1000	5000	1000	5000	
Cuttings rooted of 10 planted, transformed data†	1.835	2.140*	1.459*	1.450*	0.874*	0.235
Per cent of cuttings rooted	32.9	42.5	18.8	18.3	3.3	

* *Significantly different from control, 5% level.*
† *Data transformed to* $\sqrt{x + \frac{1}{2}}$ *basis for analysis of variance (1).*

In Table III are shown the average effects, with and without organic mercury, of indolylacetic acid and cane sugar on the length of root per rooted cutting. In the absence of sugar, indolylacetic acid increased the length of root, but the interaction between indolylacetic acid and sugar was such that the use of sugar effectively inhibited this stimulating effect of the hormone chemical.

TABLE III

ROOT LENGTHS PER ROOTED CUTTING (MM.) OF NORWAY SPRUCE CUTTINGS TREATED WITH DUSTS CONTAINING INDOLYLACETIC ACID AND CANE SUGAR

Cane sugar, %	Indolylacetic acid, p.p.m.		Means
	0	1000	
0	21	38	30
10	24	25	24
Means	23	32	

Necessary difference, 5% level, treatment means 5.2, interaction 7.4.

In Table IV are given the average effects, with and without indolylacetic acid, of organic mercury and cane sugar. In the absence of sugar, organic mercury at 50 p.p.m. reduced the length of root per rooted cutting. The addition of mercury in the presence of sugar made no difference to the extent to which root lengths were depressed. Thus, the two depressing influences together were no more effective than either alone.

TABLE IV

ROOT LENGTHS PER ROOTED CUTTING (MM.) OF NORWAY SPRUCE CUTTINGS TREATED WITH ORGANIC MERCURY AND CANE SUGAR

Organic mercury, p.p.m.	Cane sugar, %		Means
	0	10	
0	36	24	30
50	24	24	24

Necessary difference, 5% level, treatment means 5.2, interaction 7.4.

The mean length of individual roots after treatment with indolylacetic acid was 14.3 mm. as compared with 9.9 mm. without, 2.9 mm. being the necessary difference for the 5% level of significance. Treatment with 1000 p.p.m. indolylacetic acid at both dosages of cane sugar and organic mercury significantly increased the mean root length.

Discussion

Indolylacetic acid in talc dust at 1000 p.p.m. has increased the rooting of Norway spruce cuttings by about 10%. When 5000 p.p.m. of this chemical or 1000 and 5000 p.p.m. of naphthylacetic acid were used, rooting was reduced. Numerous experiments with spruces have demonstrated that it is difficult

to show improved rooting from the use of root-growth-stimulating chemicals, though injurious effects have been shown with comparative ease. A later paper will deal with the injurious effects of indolylacetic acid *solutions* on the rooting of Norway spruce cuttings.

In earlier experiments by the author (2, 3) treatment of Norway spruce cuttings with 1000 p.p.m. of indolylacetic acid in talc dust did not increase rooting significantly, though it did increase the number of roots per rooted cutting in one experiment and reduced the mean root length in two. Since the present experiment made use of material collected at the same time and propagated under closely similar conditions, a comparison of the results is justified. The sole apparent difference in procedure in the experiments was the use of cuttings with a heel in the former and plain cuttings in the present one. It is possible that differences in response to 1000 p.p.m. indolylacetic in talc may be attributed to the adhesion of a greater amount of dust to the cuttings with a heel, resulting in reduced mean root length and increased number of roots per rooted cutting. However, further experiments will be required to demonstrate whether this is so, or whether the differences in response of cuttings with and without a heel are physiological in nature.

The addition of sugar or organic mercury failed to affect the number of cuttings rooted. Some reduction in length of root per rooted cutting was noted when sugar was applied in addition to indolylacetic acid. Organic mercury also reduced root length with and without the presence of indolylacetic acid.

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THE SEXUAL BEHAVIOUR OF SEVERAL PLANT RUSTS[1]

By A. M. Brown[2]

Abstract

Experiments conducted under greenhouse conditions have shown that *Uromyces Trifolii hybridi*, *U. Fabae*, and *Phragmidium speciosum* are heterothallic. All of these rusts produce well developed pycnia. One of them, *Uromyces Fabae*, may shorten its life-cycle by omitting aecia or may produce uredia and aecia in association.

By similar experiments it has been found that *Puccinia coronata Elaeagni*, *P. Grindeliae* and *P. Xanthii* are homothallic. Ashworth's conclusion that *P. Malvacearum* is homothallic has been confirmed. In all of these rusts, single sporidia give rise spontaneously to binucleate infections. *P. Malvacearum*, *P. Xanthii*, and usually *P. Grindeliae* lack pycnia, while *P. coronata Elaeagni*, a rust described as being without pycnia, is shown to produce occasional ones.

Introduction

Since 1927, the year in which it was discovered that *Puccinia Helianthi* Schw. (11) and *P. graminis* Pers. (12) are heterothallic, the following species have been shown to possess a similar sexual behaviour: *P. triticina* Erikss. (1), *P. coronata* Corda. (2), *P. Sorghi* Schw. (4), *Melampsora Lini* (Pers.) Lev. (3), *Uromyces appendiculatus* (Pers.) Fries, *U. Vignae* Barclay (6), *Gymnosporangium Haraeanum* Syd. (17), *G. juniperi-virginianae* Schw. and *G. globosum* Farl. (19), *Puccinia Phragmitis* (Schum.) Körn. (18), *Cronartium ribicola* J. C. Fischer (23), *Uromyces graminis* (Niessl) Diet. (21), and *Puccinia anomala* Rostr. (22). Heterothallism is therefore a well known phenomenon in the rust fungi. Homothallism, however, up to the present seems to have been proved experimentally only in *P. Malvacearum* Bert. (7), and, in this case, doubt has been thrown upon the evidence (5). Jackson's (16) conclusion that, in this rust, "every infection, monosporidial or otherwise, develops one or more sori of teliospores" indicates very clearly that he found it to behave as a homothallic species.

As a result of the experiments now to be presented, it is possible to add to the above list of heterothallic species the following three rusts; *Uromyces Trifolii hybridi* (W. H. Davis) Arth., *U. Fabae* (Pers.) de Bary, and *Phragmidium speciosum* (Fries) Cooke. In addition to the heterothallic species just enumerated, three other species, *Puccinia coronata Elaeagni* F. and L., *P. Grindeliae* Peck,[3] and *P. Xanthii* Schw. are shown to be homothallic; and the finding of Ashworth (7) that *P. Malvacearum* is homothallic is confirmed.

The rust material made use of in this investigation was, with one exception, collected in the neighbourhood of Winnipeg, Manitoba. The exception was

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[3] *Arthur in his "Manual of the Rusts in the United States and Canada" indicates that on certain hosts, particularly Xylorrhiza, P. Grindeliae occasionally produces pycnia.*

P. coronata Elaeagni. Telia of this rust and roots of its aecial host, ***Elaeagnus commutata*** Bernh., for propagation purposes, were kindly provided by Dr. R. C. Russell, of the Dominion Laboratory of Plant Pathology, Saskatoon, Saskatchewan. An investigation of the sexual behaviour of ***Puccinia coronata Elaeagni*** was suggested by Dr. G. A. Ledingham, who pointed out that the absence of normal pycnial development in this variety might indicate a sexual behaviour different from that of *P. coronata Avenae* F. and L. which Allen (2) showed to be heterothallic.

Sporidial inoculation of these rusts was secured on their respective hosts by suspending germinating teliospores over seedling host-plants. This procedure, which usually provides a goodly number of well isolated monosporidial infections, is more fully outlined by Craigie (13) and Newton and Johnson (20). The procedure followed in diploidizing haploid infections of the pycnium-producing rusts by means of pycnial-nectar transfers, and by permitting haploid and diploid (uredial) infections to unite, has previously been described by Craigie (12) and Brown (9) respectively.

The experiments were, for the most part, conducted during the winter months, and during that time insects that might transfer nectar from one infection to another were practically absent from the greenhouses. However, in order to prevent any possibility of such a transfer, each plant was enclosed in a screen-wire cage.

Heterothallic Species

Uromyces Trifolii hybridi

As a result of suspending germinating teliospores of *U. Trifolii hybridi* over seedling plants of *Trifolium hybridum* L., 60 well-isolated haploid infections were secured. Of this number, 20 infections were allotted as controls; to 20 others, composite pycnial nectar collected from infections not used in the experiments was applied; and, near the periphery of 15 others, urediospores of this rust were sown. The plants bearing these 15 infections together with the remaining five haploid infections were enclosed in a moist chamber for 48 hr., a procedure usually followed in uredial inoculation practice. The five haploid infections served as a control for the other 15.

Of the 20 infections to which pycnial nectar was applied, 19 produced aecia within seven days following the application of nectar, but the 20 control infections, *i.e.*, those to which no nectar was applied, were still haploid at the termination of the experiment two weeks later. All of the 15 infections, around the periphery of which urediospores were sown, produced aecia in from 10 to 12 days following the uredial inoculation (Plate I, A.). The five control infections, however, around the periphery of which no urediospores were sown, remained haploid.

Uromyces Fabae

Following the method employed to secure haploid infections in the experiments just mentioned, 60 well-isolated monosporidial infections of the strain of *U. Fabae* inhabiting *Lathyrus venosus* Muhl., were secured on seedling hosts

of *L. ochroleucus* Hook. and *L. venosus*. When these infections were 16 days old, there was applied to 50 of them composite pycnial nectar drawn from infections other than those used in the experiment. Seven days after the transfer of nectar, 49 of the 50 infections receiving it produced aecia. Within three days after the appearance of aecia, 17 of the infections produced uredia in association with the aecia (Plate I, B). The infections to which no nectar had been applied remained haploid during the course of the experiments.

Thirty compound infections, each formed by the union of two monosporidial infections, were under observation concurrently with the monosporidial infections just mentioned. When these infections had completely coalesced, 17 of the 30 produced uredia. The uredia appeared in one component several days in advance of uredia in the other. No aecia developed in any of the infections, and the 13 infections that did not produce uredia remained in the haploid condition. It is improbable that diploidization of the 17 infections producing uredia was effected by means of pycniospore intervention, that is to say, by the interflow of pycnial nectar from one component to the other, for, during the winter months, pycnial nectar was sparingly produced, and what was produced usually collected in the centre of each infection.

In another experiment, urediospores were sown near the periphery of each of ten isolated monosporidial infections. Ten days or so later, when the diploid (uredial) and haploid infections had coalesced, all of the ten formerly haploid infections produced uredia and aecia in association and at the same time. Two haploid infections near the periphery of which no urediospores had been sown remained unchanged.

Perhaps in this rust the short-cycling tendency just noted is not an unusual phenomenon. It seems that, as early as 1863, de Bary (8) encountered in this rust a similar behaviour, for he states that "in some instances the same mycelium which produces aecia gives rise to a few isolated uredia".

Phragmidium speciosum

Small plants of a species of wild rose (*Rosa blanda* Ait.) were inoculated in the greenhouse with sporidia of *P. speciosum*. Altogether, 150 haploid infections were secured. In addition to these, there were 15 compound infections, each formed by the coalescence of two haploid infections.

Following the method already referred to, composite pycnial nectar was applied to 100 of the haploid infections. The other 50 were kept as a control. Ten days after the nectar was transferred, 91 of the 100 infections receiving it produced aecia. The control infections to which no nectar was applied remained unchanged. Of the compound infections, 8 of the 15 produced aecia.

Diploidization of haploid infections by means of uredial infections was not possible in this rust, as urediospores are wanting. Repeated attempts to diploidize haploid infections by sowing aeciospores of this rust near their periphery failed. The aeciospores did not infect the leaves of the host plant, although they germinated freely in hanging drops of tap water. As this rust

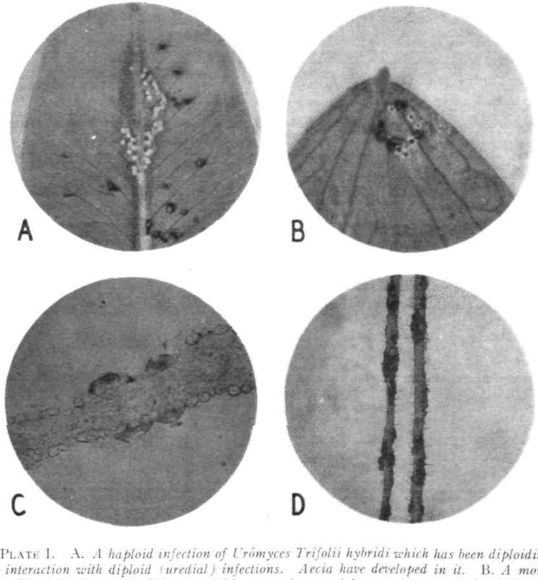

PLATE I. A. *A haploid infection of Urômyces Trifolii hybridi which has been diploidized by interaction with diploid (uredial) infections. Aecia have developed in it.* B. *A monosporidial infection of U. Fabae to which composite pycnial nectar was applied. It became diploidized and produced aecia and uredia in association.* C. *A cross section of an infection of Phragmidium speciosum on a rose leaf, showing pycnia on both the upper and lower surfaces. × 100.* D. *Telia of P. speciosum on rose twigs. The infections earlier produced pycnia and aecia. One-half natural size.*

is autoecious, the behaviour of these aeciospores is of interest. Perhaps they might be capable of infecting some other species of *Rosa*, or a species belonging to an entirely different genus. This possibility was not investigated on account of the time of year at which the experiments were made. It should be pointed out, however, that in this rust the telia arose in the same infections that produced the aecia, and not as a result of aeciospore infection.

The pycnia of *P. speciosum* are, in general, developed equally abundantly on the upper and lower surfaces of infections on rose leaves (Plate I, C). Pycnial paraphyses are wanting, but flexuous hyphae and pycniospores are present and nectar is copious. Telia can readily be obtained on the leaves under greenhouse conditions, but under natural conditions they are found mostly on the young stems of the host (Plate I, D), for defoliation generally occurs in Western Canada before telia can fully develop in leaf infections.

When the pycnial nectar of 29 isolated monosporidial leaf infections was intermixed on the lower surface only, 26 infections produced aecia six days afterwards. Consequently, in this rust, nectar transfers, when made on the under-surface of leaf infections, are as effective in promoting aecial production as when made on the upper surface.

From the results of the experiments described above, it is concluded that *Uromyces Trifolii hybridi*, *U. Fabae* and *Phragmidium speciosum* are heterothallic, and that, in addition, *Uromyces Fabae* may shorten its life-cycle occasionally by omitting the aecial stage.

Homothallic Species

Puccinia coronata Elaeagni

The uredial and telial stages of *P. coronata Elaeagni* occur on a variety of *Calamagrostis elongata* (Kearney) Rydb.,[1] and the aecial stage occurs on *Elaeagnus commutata* Bernh.

During the winter months of 1936–7, young plants of *E. commutata* were inoculated with sporidia of *Puccinia coronata Elaeagni* by the method of inoculation previously mentioned. Each plant was but lightly inoculated, so that, as a rule, only two or three infections occurred on any plant, and usually only one infection was present on any leaf. The inoculations were not all made on the same date, but all were made during the winter months (December to March). At that time, as already mentioned, practically no insects were present in the greenhouse, but as a precautionary measure each inoculated plant when taken from the moist chamber was placed in a screen-wire cage. This precaution was taken as Allen (5) had found conidia in infections of *P. Malvacearum* and had suggested that these might be transferred by insects from one infection to another, or to the immediate vicinity of another infection, and, on germination, might enter the leaf tissue and produce

[1] *Fraser and Ledingham (15) state that the late Dr. M. O. Malte, while Dominion Agrostologist, considered the grass host of this rust to be a dry land form of Calamagrostis elongata, and for this reason that name is adopted here.*

mycelia that would interact with the mycelium of the original infection and diploidize it. There was, therefore, the possibility that, in monosporidial infections of *P. coronata Elaeagni*, similar conidia might be present and that, even in the absence of pycnia and pycniospores, diploidization of such infections might occur through the agency of insects. In this connection, it may be stated that during the course of the experiments no insects were observed on the plants.

Altogether, 51 infections were kept under observation. They were watched closely from the time of their earliest appearance, and in none of them was there any evidence that they were of bisporidial origin. Consequently, each of them was regarded as a monosporidial infection. In none of them could pycnia or pycnial nectar be detected with the aid of a strong hand-lens, yet 49 of the 51 infections produced aecia spontaneously within 36 days from the date of inoculation.

There was considerable irregularity in the age at which the different infections produced aecia. A few produced them ten days after inoculation, at which age the infections were quite small and only two or three aecia were present in each infection. In the remaining 26 days during which the infections were kept under observation, some of the infections formed aecia at one age, others at another. Ultimately as already mentioned, only two of the 51 infections failed to produce aecia, and even these two might have produced aecia had they been retained longer. The spontaneous diploidization of these infections indicates that *P. coronata Elaeagni* is homothallic.

Why some infections required a much longer time than others to produce aecia is not understood. The infections developed aecia in much the same haphazard manner as would haploid infections of *P. Helianthi* if left exposed to the occasional visit of insects; yet, in a rust like *P. Helianthi*, if no insects were present, as was the case in this experiment, most of the infections at least would have remained undiploidized. No such delay, however, occurs in the production of telia by *P. Malvacearum*, another homothallic rust, as has just been mentioned in the production of aecia in *P. coronata Elaeagni*.

Although this rust has been described as lacking pycnia (15), abortive pycnia (Plate II, A) occasionally are present. These can only be detected by sectioning the infections. They are composed entirely of hyphae, and show no evidence of pycniospores. Pycnial nectar is not produced, and paraphyses are absent. Besides, no flexuous or "surface" hyphae protruding from the infections were observed, so that it seems impossible that conidia, such as those mentioned by Allen (5) as occurring in *P. Malvacearum*, were present. There was not the possibility, therefore, of newly formed conidia causing infections near existing infections and thus affording an opportunity for the interaction of two mycelia of opposite sex. The mechanism by which diploidization can be effected by insects seems to be absent in this rust.

The sporidia of *P. coronata Elaeagni* when first abstricted are uninucleate, but shortly afterwards, even before they germinate, the majority of them

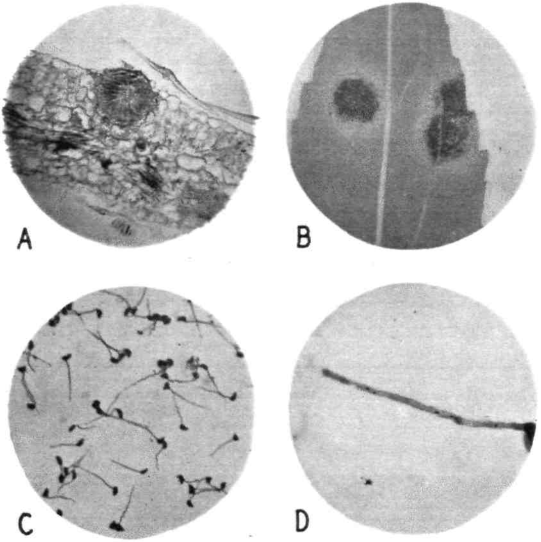

PLATE 11. A. *Cross section of an infection of Puccinia coronata Elaeagni, showing an abortive pycnium.* × *100.* B. *A single and a compound infection of P. Xanthii. Both have produced telia simultaneously. About natural size.* C. *Germinating sporidia of P. Grindeliae.* × *200.* D. *A germinating sporidium of P. Grindeliae showing nuclei in various stages of division.* × *600.*

become binucleate. Occasionally when sporidia are about to germinate, some of them may contain three or even four nuclei.

As far as observed, the hyphae of young infections and their aecial primordia are uninucleate. The binucleate condition seems to appear first just prior to the differentiation of the first stalk cells of the incipient aecium, but how it is brought about was not definitely determined. As, in an examination of many sections, no cell fusions were observed, it seems reasonable to conclude that the binucleate condition arose as a result of nuclear division without cross wall-formation.

Puccinia Malvacearum, *P. Grindeliae*, and *P. Xanthii*

An investigation was also made of the sexual behaviour of *P. Malvacearum*, *P. Grindeliae*, and *P. Xanthii*. The teliospores of these rusts germinate readily without undergoing any prolonged dormancy period, so that, during the winter season of 1937–8, successive sporidial inoculations of their respective hosts were made with each of the three rusts. Shortly after the infections became visible, an examination was made with a strong hand lens to determine which infections were monosporidial and which bisporidial in origin, in order that their identity would not be confused.

One thousand and ten monosporidial infections of *P. Malvacearum* were obtained on *Althaea rosea* (L.) Cav. and *Malva rotundifolia* L.; 150 of *Puccinia Grindeliae* on *Grindelia squarrosa* (Pursh) Dunal; and 350 of *Puccinia Xanthii* on *Xanthium commune* Britt. All of these infections were well separated, and there is little doubt that all, or practically all, were monosporidial in origin. When from 10 to 12 days old, all of the infections produced telia spontaneously.

Besides the many monosporidial infections just mentioned, 650 compound infections, each formed by the coalescence of two monosporidial infections, were randomly selected from the three rusts for observation. All of these infections produced telia simultaneously in both components and within the same length of time as that required for telial production in the monosporidial infections. Both components of each compound infection produced telia spontaneously at their centres, as if each component had become binucleate independently of the other component (Plate II, B). This observation was confirmed later by an examination of microtome sections cut from several compound infections that had just produced telia. The sections showed binucleate mycelia (at the centres of the two components) separated by an intervening area of uninucleate mycelium. No hyphal fusions were detected, and there was no evidence to indicate that nuclear interchange had taken place between the components. The behaviour of these compound infections is in striking contrast to those of a heterothallic rust, such as *Puccinia Helianthi*. In the latter rust, aecia develop in about one-half of the compound infections (10, 11, 12). Moreover, if, in a compound infection having components of opposite sex, the intermixing of the pycnial nectar of the two components is prevented, one component will invariably produce aecia in advance of the other component (10). In the three rusts now under discussion, all of the compound

infections produced telia, and, without exception, produced them simultaneously in both components. From these results it is evident that these three rusts are homothallic.

In these three rusts the promycelium produces four uninucleate sporidia, which soon after abstriction germinate readily in a film of moisture (Plate II, C). Nuclear division occurs rapidly after germination takes place and two or more nuclei, in various stages of division, may be seen in the germ tube (Plate II, D). The mycelium of monosporidial infections is definitely uninucleate, but when the infections are about eight days old, the binucleate stage, as already indicated, usually appears near their centres.

From an examination of a large number of sections cut from five- to seven-day-old infections of the three rusts, it was evident that the binucleate condition regularly arises as a result of nuclear division without cross-wall formation, and not by nuclear migration. This finding seems to be in agreement with that of Ashworth (7), although she reported infrequent nuclear migrations.

The binucleate cells then become club-shaped (Plate III, A) and the two nuclei in each divide. A cross wall is then laid down, dividing the club-shaped cell into an apical and a basal part, each part containing two nuclei that fuse into one as the spore matures (Plate III, B). Nuclear irregularities, however, sometimes occur and club-shaped cells (Plate III, C) containing three or more nuclei may from time to time be encountered.

All of the rusts so far known to be heterothallic, including the three of the present investigation, possess well developed pycnia. Only four rusts are known to be homothallic, the one reported by Ashworth (*P. Malvacearum*) and the three of the present investigation. Of these four, *P. Malvacearum* and *P. Xanthii* lack pycnia entirely, *P. coronata Elaeagni* produces occasionally abortive pycnia, but generally they are absent, and *P. Grindeliae* produces pycnia only on one of its hosts (*Xylorrhiza*). It would appear from this limited evidence that the presence of pycnia in a rust would indicate that the rust is heterothallic, and that the absence or underdevelopment of pycnia in a rust would indicate that the rust is homothallic. The fact that *P. coronata Avenae* is heterothallic and possesses well developed pycnia while *P. coronata Elaeagni* is homothallic and develops occasionally abortive pycnia, would seem to support the assumption that homothallic rusts may have been at some stage in their development heterothallic. If the absence or underdevelopment of pycnia in these homothallic rusts be regarded as evidence of degeneration, the assumption seems to receive further support.

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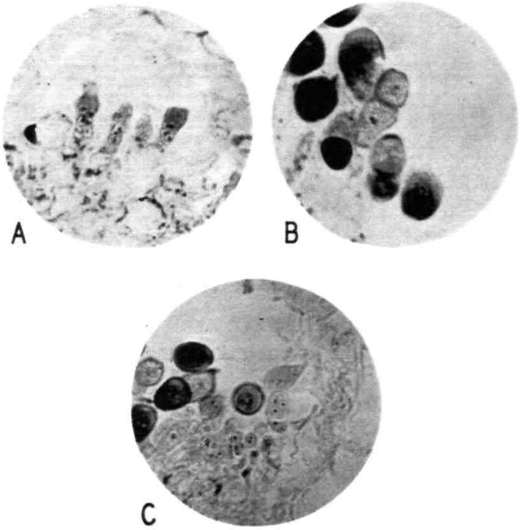

PLATE III. A. *Part of a section cut from a monosporidial infection of Puccinia Malvacearum showing four club-shaped cells. In the two cells at the left and the one at the right, one nucleus has divided and the two daughter nuclei have drawn apart; the other nucleus is still undivided. The two nuclei of the cell third from the left have not yet begun to divide.* × *350.* B. *A maturing teliospore of P. Malvacearum with the two nuclei in the lower cell in close contact.* × *600.* C. *A section of a young telium of P. Malvacearum, showing cells with one, two and three nuclei.* × *550.*

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HISTOLOGY OF SOME PHYSIOLOGICAL DISORDERS OF THE APPLE FRUIT[1]

By Mary MacArthur[2]

Abstract

The histological similarities and differences between the boron-amenable disorders, internal cork, corky core, and drought spot, and the non-boron-amenable disorder, bitter pit, are presented. Blotchy pit is discussed as a border-line type, since one end of a graded series is similar to internal cork and the other is indistinguishable from bitter pit. On the basis of histology water core is not placed in either of the two groups.

Starch retention, in localized or diffuse necrotic areas, is common to both groups of disorders. External papillations occur on the walls of cells in close proximity to the lesions. Abnormal meristematic activities coexist with boron deficiency. These are (1) a cork cambium partially or completely walling off a lesion, (2) massed linear cells, heavily papillated, and (3) reactivated individual cells or groups of cells. All three types of abnormal cells may be present in internal cork and drought spot, but no cork cambium occurs in corky core.

The currently used diagnostic characters of the physiological disorders of the apple are based almost exclusively on the gross morphology of the lesions. The present study was undertaken in an attempt to differentiate the various disorders from a histological point of view. It is realized that an adequate inquiry into the problem should comprise a detailed study of the fruit throughout the season for the purpose of correlating growth with initiation and progress of histological changes. While this study is preliminary, sufficient progress has been made on general anatomical characters to justify its presentation.

Methods

Collections were made of six disorders in 15 varieties of apples in orchards of Ontario, Quebec, and Nova Scotia. More than 50 collections were made during the late summer and autumn of 1938.

Blocks of affected apple tissue up to 1 cc. in volume were killed and fixed in formalin-acetic-alcohol. The samples were dehydrated in an *n*-butyl alcohol series and embedded in paraffin, m.p. 53 to 55° C. Sections 10 to 15μ thick were made, and various stains, such as Haidenhain's iron alum hematoxylin, crystal violet and orange G, safranin and fast green, were used. In addition, the presence of suberized tissue was tested with Sudan IV, the sections being mounted in glycerin jelly to avoid the de-staining concomitant with the use of xylol-balsam. The presence of pectin substances was investigated by the use of Ruthenium red and by the Prussian blue precipitate formed on treatment of such substances with ferrous chloride, potassium ferrocyanide and hydrochloric acid.

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The disorders examined were Internal cork, Corky core, Drought spot (Superficial cork), Bitter pit (Tree pit, normal type), Blotchy pit (Tree pit, blotchy type) and Water core.

Observations

Internal Cork

The three varieties—Fameuse, Ontario, and Delicious—from which samples were taken all showed varying degrees of the typical pebbly surface associated with severe cases of the disorder. A second diagnostic character of internal cork is the greenish tissue delimiting a lesion. Frequently this greenish tinge is absent, particularly in later-formed cork. Such lesions then have the gross appearance of bitter pit. Both types of lesions were examined.

Whether or not the peripheral coloration of the lesion is present, definite lesions of internal cork exhibit microscopically either a more or less continuous delimiting cambium with its derived tissues (Fig. 1), or a closely compacted growth of abnormal linear tissue (see following). Mix (6) figures a ladder-like arrangement of nearly rectangular cells around the "corky" portion. He states that these are healthy cortex cells. Tests with Sudan IV indicate that the cambium is a cork cambium. In the suberized layer, the phellem is more extensive than the phelloderm. The designation "cork" in this disorder is not a misnomer. The lesion was originally termed "cork" because the brown spongy, internal necrotic area superficially resembles cork. However, there is no suberized tissue in this region.

External to the phellem are numerous "reactivated" cells. The walls of such cells are definitely thinner and the nuclei are prominent. Interspersed among the reactivated cells, and often filling entirely the intercellular spaces, is a second type of abnormal cell. This abnormal tissue (Fig. 2) is composed of attenuated cells. The writer has observed numerous cases of the initiation of such tissue by *(a)* division of a cortical cell, and *(b)*, the linear growth of phellem cells. In *(a)*, a daughter cell from the division projects into the intercellular space and linear growth from subsequent divisions is continued at the distal end. Branching occurs frequently. Linear growth from the outer phellem cells is similar. The tissue resembles the transversely septate hyphae of the Ascomycetes, but the cells are many times as large, and are heavily papillated. Although this tissue is not discussed in the text, it is pictured by Verner (12, Plate 5B). In this plate, it appears as if the abnormal tissue arises in close relation to the cork cambium, which cuts off the hypodermal tissue in cracking of apples. It is similar to the tissue extending into the locules in woolly core of apples (10). Many of the apples examined in this study were affected with woolly core, but there was no correlation between the presence of such tissue arising from the split cartilaginous endocarp and abnormal linear cells in other tissue.

Both abnormal types of apple cells contain starch grains which are significantly smaller than the starch grains of normal cells. These smaller starch

grains do not stain as deeply with either crystal violet or iodine-potassium-iodide.

The walls of normal-appearing cells contiguous to the lesions are also papillated. The prominence of the papillations decreases with increasing distance from the lesion. The papillae may range from slight extensions of the wall to finger-like or mushroom-shaped protrusions. It is believed that they are concentrations of the middle lamellae. They are always external and stain conspicuously with hematoxylin.

The necrotic area of the internal cork lesion is composed mainly of collapsed cells with imprisoned starch grains. Closely but unevenly applied to the inner surface of the generally ruptured cell is a brownish, gummy or mucilaginous substance. Micro-chemical tests demonstrate that the mass is of a pectic nature. However, it is different from that found in the walls, since the walls of cells in the necrotic area stained with Prussian blue, which gradually faded to a paler blue, while the Prussian blue stain of the mucilaginous masses slowly changed to a green, and finally to a yellow-green.

Vascular bundles which penetrate or approximate the lesion are affected. Plugging in the form of a finely-granular substance appears in the phloem cells. *In addition, a second disorder may be present in the same tissue.* This is in the form of masses that are yellow in unstained section and that stain deeply with hematoxylin. These masses are found between normal phloem cells. This may indicate gummosis of part of the phloem, or formation of intercellular spaces by splitting of the middle lamellae and subsequent secretion of pectic substances, which finally fill the space. It is somewhat similar to the disorder found in the phloem of the potato affected with leaf-roll (1). In many cases the xylem of the bundle is also affected. The secondary thickening of the spiral tracheids is coated with an undetermined substance which prevents the clear staining reaction effected with normal cells.

Corky Core

The varieties McIntosh Red, Scarlet Pippin, Ben Davis, Baldwin, and Northern Spy were affected with this disorder. The gross morphological character of the disorder is a slight browning in the pith area, usually in the regions to the right and left of the dorsal carpel bundle and, in more severe cases, browning of the whole pith (core region) centripetally from the main fibrovascular bundles. The more severely affected fruits may have lysigenous cavities formed by the collapse and separation of large groups of cells.

Microscopically the lesion is diffuse. The cells are more or less tangentially collapsed. Starch grains are imprisoned between the approximated cell walls. Starch may be completely absent from the surrounding tissue, except in those younger parenchyma cells that are added to the ground tissue by the cambial activity of the larger fibrovascular bundles. When reactivated cells are present, they are cuboidal in shape. A long, narrow pith cell which is stimulated divides several times. Thin cross walls are formed, and the final result is a pith cell divided into a linear series of cuboidals. Abnormal linear growth similar to that found in internal cork is always present, but is some-

what diffuse (Fig. 3) and may be sparse. This abnormal tissue is found in the intercellular spaces contiguous to the necrotic area. It may enter the lacunae formed on the collapse of cell groups. As with internal cork, the walls of the affected cells stain more deeply. Papillae are present, but are not as conspicuous as those accompanying internal cork. Small quantities of a gummy material similar to that in the previous disorder is closely applied to the inner side of the ruptured or collapsed cell wall.

Drought Spot or Superficial Cork

This disorder was examined in the varieties McIntosh Red and Ben Davis. The gross diagnostic character of the disorder is a rugosity of certain localized areas. Cracks may develop and the fruit may become malformed. Later drought spot may have a russetted appearance or simply a cuticular checkering. However, in Ben Davis definite lesions are visible through the epidermis. Such lesions may or may not involve the surface. In all cases, the outer tissues primarily are affected.

As normal growth of the apple proceeds, the epidermal and hypodermal cells become tangentially elongated (11). If these cells are affected early, they are rounded, have a heavily cutinized epidermis and a thick-walled hypodermis. The rounded hypodermal cells are often loosely arranged, since they are radially disrupted by the tangential torsion and the radial pressure of the subjacent growing tissue. In light cases of the disorder, the hypodermal cells are severely flattened but not separated. The lumen is a narrow slit filled with a dark-staining, finely granular deposit. Since the outer tissues in drought spot areas have little extensibility, the continued growth of the underlying tissues causes cracking. It is in these more severe cases that a cork cambium is initiated in the outer cortex (Fig. 4). Epidermis and hypodermis are then sloughed off irregularly. Some of the cortical cells collapse and imprison starch grains. Throughout the intercellular spaces and centripetally from the outer limiting periderm layer, abnormal linear tissue is interspersed. In the hypodermal area, the papillae of the cell walls may bridge the narrow intercellular spaces and so give the appearance of a lattice.

In Ben Davis the lesion closely approaches internal cork in appearance (Fig. 5). The cambial layer is less continuous but the abnormal tissue is much more extensive. Reactivated cells of both cuboidal and filler type are present. The central region of the lesion is composed of radially collapsed cells with imprisoned and generally clumped starch grains. Such grains are embedded in a matrix probably composed of breakdown products of the protoplasm. Serial sectioning discloses that a fibrovascular bundle of the cortex is in contact with a lesion. Some of the sieve tubes and parenchyma of the bundle show collapse. Often the bundle cells are forced apart by abnormal linear growth. In addition, the bundle cambium may initiate cuboidal parenchyma. Papillae occur on the cell walls and are more conspicuous in close proximity to the lesion.

Bitter Pit (Tree pit, normal type)

Bitter pit was examined in Cooper's Market, Stark, Blenheim, Red St. Lawrence, Baldwin, and Northern Spy. The surface lesions of these samples were uniformly coloured, red, brown or green, were up to 7 mm. in diameter, slightly to not at all depressed, and involved tissues external to the main fibrovascular bundles. In some of the samples, other disorders such as corky core or water core were present.

When starch grains are equally distributed throughout the external tissues and when there is yet no breakdown, cell walls in certain areas stain intensely with the Prussian blue precipitate in tests for pectin substances. Since the necrotic areas of typical bitter pit exhibit a similar but stronger staining reaction, it is believed that the above reaction indicates an early stage of the disorder. These same areas also stain heavily with Ruthenium red. Later, a few cells collapse. With further development collapsed cell walls withdraw from centralized areas, leaving small to larger lacunae, and starch grains are held between collapsed walls (Fig. 6). The starch is usually embedded in an amorphous substance which is probably the dead and discoloured protoplasmic contents of the affected cells. At this stage there may be more starch in the surrounding healthy cells. Still later, with starch hydrolysis accompanying the maturation of the cells, starch disappears from the healthy tissue and the starch content of the necrotic areas becomes conspicuous.

These observations on the starch are believed to be evidence that normal starch metabolism is disrupted early, and that the presence of starch grains in the necrotic areas alone at later stages is merely incidental, since by their location in affected areas they were outside the sphere of normal metabolic, and therefore hydrolytic, activity. This is in accordance with the views of Smock (8) concerning the persistence of starch in bitter pit, and again, "starch persistence may be concomitant with the trouble rather than causal." (9).

In most cases of bitter pit examined, serial sections revealed that the lesion was in contact at some point with a fibrovascular bundle (7, 9). Only when the bundle passed directly through a lesion was definite disorganization of the bundle evident. In transverse sections of bundles at the periphery of lesions, affection of the sieve tubes and parenchyma similar to the collapse observed in drought spot was noted. It is impossible without the investigation of earlier collections to determine whether this disturbance is of primary importance or is to be considered an effect of the disorder.

Neither abnormal linear growth, reactivated cells, nor a secondary cambium formation was observed in any sample of bitter pit when bitter pit alone was present. The cell walls were papillated and, as with the disorders previously described, the papillae were well defined near the lesions.

Blotchy Pit (Tree pit, blotchy type)

Blenheim, Stark, Delicious, Tolman Sweet, and Deacon Jones affected with this disorder were examined. Macroscopically the lesions approximate bitter pit in appearance, but are larger and are dark green mottled with brown.

Anatomically, there are two extreme types of blotchy pit. Type I (Fig. 7) closely resembles internal cork, and Type II (Fig. 8) is identical in appearance with bitter pit. Between these two extreme types gradations are found. In Type I a discontinuous cambium defines the lesion. At the periphery of the lesion where cambial activity is not present, the intercellular spaces and lacunae of collapsed cell groups are packed with abnormal linear tissue. Reactivated cells, mainly of the filler type (Fig. 2) are also present. In Type II there is neither cambial activity nor abnormal growth.

In the necrotic areas of both types there is cell collapse, lacunar formation and retention of starch grains. The dead, coagulated protoplasm adheres to the walls. The walls of living cells are always papillated.

In the intergrade types, a cork cambium cuts off external cork in a shallow hypodermal lesion, or, when cork is not present, the sub-epidermal cells are radially flattened, tangentially elongated and thick-walled. The narrow lumen is filled with a dark-staining material. Some few abnormal linear cells are found in the intercellular spaces of the outer cortex.

Water Core

Blenheim, Red St. Lawrence, Northwestern Greening, Delicious, and Tolman Sweet were affected with water core. Watery injection of the tissues is the diagnostic character. When the evidence is external, the skin is darker in colour, the injected areas being seen through the epidermis.

When the disorder was located in the core region, the primary fibrovascular bundles were involved. Cortical vasculars arising from the affected primaries exhibit a water-logged sheath (Fig. 9). Large water-core areas may finally show a breakdown of the soggy type (Fig. 10).

Microscopically, no abnormal growth occurs when water-core alone is present. A few weakly-staining starch grains are found, but most of the starch has become hydrolyzed. The middle lamellae stain lightly with hematoxylin, evidence that the cell wall pectins are changed. Of the bundles, only the parenchyma cells of the sheaths are affected. These are shrunken and collapsed. The cell walls are not papillated.

For convenience of comparison, the anatomical characters of the disorders investigated are arranged in a table.

Discussion

The physiological disorders of the apple fruit that occur on the tree are of two classes, boron-amenable and non-boron-amenable. Of the disorders examined in this study, internal cork, corky core, and drought spot are alleviated by the use of boron in the nutrition of the tree, but bitter pit is not affected. Most of the nutritional work done on blotchy pit indicates that it is a form of bitter pit, but Carne and Martin (2), in their boron treatments of Cleopatra, state that this variety apparently responded to liquid injections of boric acid. However, they doubted the significance of the results in view of the highly significant difference in fruit size. Many other experiments of

TABLE I

A SUMMARY OF THE ANATOMICAL INVESTIGATION OF SOME DISORDERS OF APPLES

Disorder	Variety	Cell collapse	Starch retention	Papillae	Abnormal growth		Cambial activity
					Linear	Reactivated	
Internal cork	Fameuse	+	+	+	+	+	+
		+	+	+	+	+	+
		+	+	+	+	+	+
		+	+	+	+	+	+
	Ontario	+	+	+	+	+	+
		+	+	+	+	+	+
	Delicious	+	+	+	+	+	+
Corky core	McIntosh	+	+	+	+	+	−
		+	+	+	+	+	−
		+	+	+	+	−	−
		+	+	+	+	−	−
		+	+	+	+	−	−
	Ben Davis	+	+	+	+	+	−
	Baldwin	+	+	+	+	−	−
		+	+	+	+	−	−
	Northern Spy	+	+	+	+	−	−
	Scarlet Pippin	+	+	+	+	−	−
Drought spot	Ben Davis	+	+	+	+	+	+
		+	+	+	+	+	+
		+	+	+	+	+	+
		+	+	+	+	−	−
		+	+	+	+	−	−
	McIntosh	+	+	+	+	+	−
		+	+	+	+	−	−
		+	+	+	+	−	−
		+	+	+	+	−	−
Bitter pit	Cooper's Market	+	+	+	−	−	−
		+	+	+	−	−	−
	Baldwin	+	+	+	−	−	−
		+	+	+	−	−	−
	Blenheim	+	+	+	−	−	−
	Northern Spy	+	+	+	−	−	−
		+	+	+	−	−	−
	Red St. Lawrence	+	+	+	−	−	−
	Stark	+	+	+	−	−	−
		+	+	+	−	−	−
Blotchy pit	Blenheim	+	+	+	+	+	+
		+	+	+	+	−	−
	Stark	+	+	+	+	+	+
		+	+	+	+	+	−
		+	+	+	+	+	−
		+	+	+	+	−	−
		+	+	+	−	−	−
	Delicious	+	+	+	+	+	−
	Tolman Sweet	+	+	+	+	+	−
		+	+	+	−	−	−
		+	+	+	−	−	−
	Deacon Jones	+	+	+	−	−	−
Water core	Blenheim	−	−	−	−	−	−
	Delicious	−	−	−	−	−	−
	N. Greening	−	−	−	−	−	−
	Red St. Lawrence	−	−	−	−	−	−
	Tolman Sweet	−	−	−	−	−	−

the same nature are inconclusive because of the absence of the disorder on both treated and control trees.

Internal cork, corky core, and drought spot all showed abnormal growth. None was found in bitter pit, while blotchy pit was intermediate. One type of lesion is similar to bitter pit, others approach the various boron-amenable lesions. The difficulty of obtaining definite results in blotchy pit may be due, in part at least, to this difference in types.

Living tissues in their metabolic activity pass through three phases. Fig. 11 illustrates the normal cycle of activity. It is known that internal cork, drought spot, and possibly corky core appear earlier in the developmental history of the fruit than bitter pit. It is suggested that the first three disorders appear in phase 1, when anabolic activity is predominant; that blotchy pit of the several types appears in phases 1, 2 or 3, and that bitter pit appears only in 3.

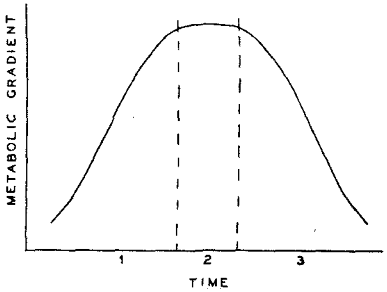

FIG. 11. *Theoretical curve of metabolic activity.*

The majority of the tissues of the fruit are fundamental in nature, and are capable of stimulation to meristematic activity, provided that the cells are in the anabolic stage of metabolism. Stimuli may be varied to call forth such a reaction. Intumescences of cabbage which show cambial activity similar to that found in internal cork, drought spot and certain blotchy pit lesions may be caused by a condition common to exposure to acid or alkaline vapours, to freezing, to drying by excessive transpiration from exposed areas, to aphis punctures, to breaking the leaf surface or to injections of water that loosen the epidermal layer (5). Haberlandt (4) attributes a similar growth in storage cells of the potato tuber to a growth-producing enzyme in the companion cells of the phloem. Gilbert (3) found a cork cambium surrounding the necrotic areas of potato tubers affected with net necrosis. He believed the meristematic activity was due to an accumulation of soluble nutrients at the blocked walls.

Therefore, all such abnormal meristematic activity is initiated by some stimulus. It is conceivable that deficiency of boron initiates a physiological change in localized areas. The death of the cells of such areas and the meristematic activity of contiguous cells in the anabolic phase are links in the chain of reactions set up by the disrupted metabolism. In bitter pit, the factors concerned with the initiation of necrosis differ, for boron applications do not reduce the incidence of the disorder. Meristematic activity is not an adjunct of lesion formation in bitter pit. Therefore, the metabolic stage of the tissue is a factor which must be considered. There is a possibility that boron deficiency is the stimulus in some types of blotchy pit.

Starch retention in greater or lesser quantity in all necrotic areas indicates changed localized metabolism. Ergastic inclusions as starch grains are thus thrown outside the sphere of normal hydrolytic activity. The size differences between such starch grains and those of abnormal tissue are probably due mainly to the relative physiological adjustment between size of cell and size of starch grain.

It is difficult to determine whether the partial disorganization of the bundles in contact with lesions is primary or secondary. All lesions penetrated by fungus or caused by insects contained numerous globular masses of an undetermined substance. Such masses were conspicuous in the parenchyma of the proximal bundles.

The study of the disorders occurring on the tree and their relation to the storage disorders is being continued.

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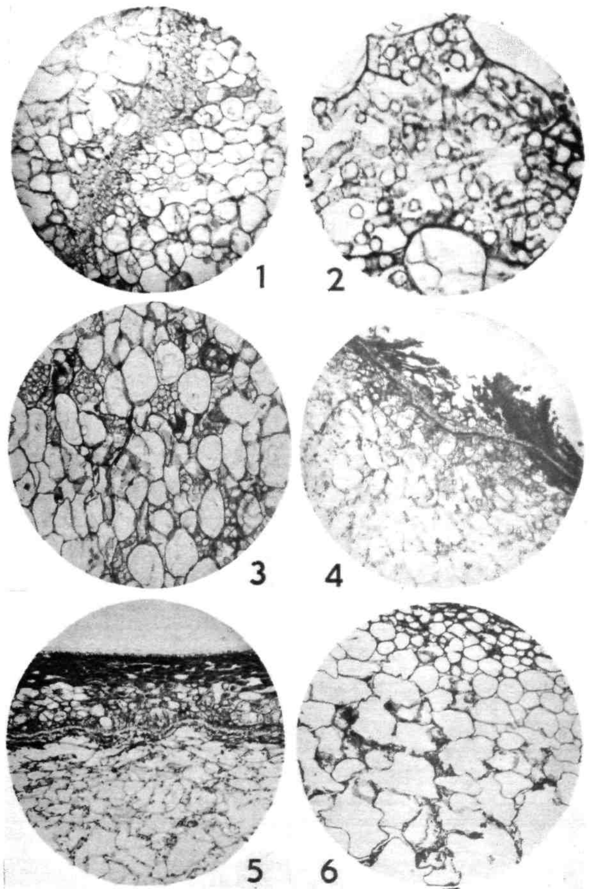
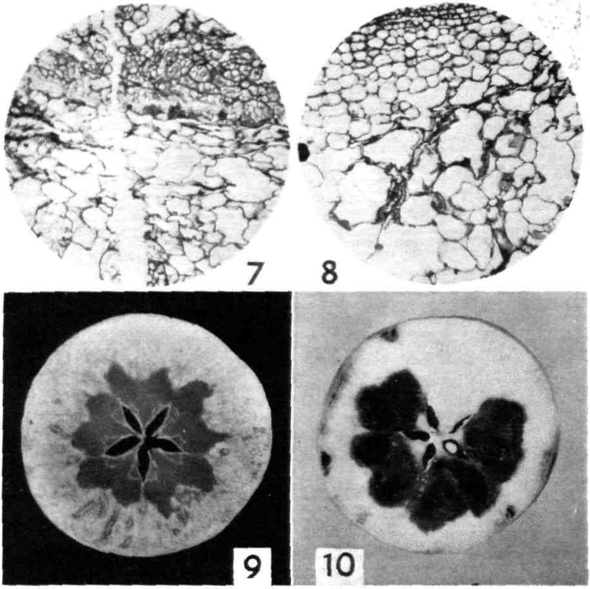


FIGS. 1 – 6. FIG. 1. *Internal cork, Ontario. Left, necrotic tissue; centre, cork cambium; right, "reactivated" and normal cells, interspersed with abnormal linear tissue.* $\times$ *80.* FIG. 2. *Abnormal linear cells with conspicuous papillae. Reactivated cell of "filler" type at bottom of figure.* $\times$ *215.* FIG. 3. *Corky core, Northern Spy.* $\times$ *80.* FIG. 4. *Drought spot, McIntosh Red. Cambium in outer cortex. The scattered dark spots are starch grains.* $\times$ *80.* FIG. 5. *Drought spot, Ben Davis, showing cork cambium. Necrotic tissue in lower part of figure.* $\times$ *80.* FIG. 6. *Bitter pit, Baldwin. Note retention of starch and papillated walls. Compare with Fig. 8.* $\times$ *80.*



FIGS. 7–10. FIG. 7. *Blotchy pit, type 1. Top, abnormal linear cells; upper centre, cambium; lower, necrotic tissue. Compare with Fig. 1.* × *80.* FIG. 8. *Blotchy pit, type 2. Deacon Jones. Note similarity to bitter pit, Fig. 6.* × *80.* FIG. 9. *Water core, Red St. Lawrence.* FIG. 10. *Water core breakdown, soft type. Tolman Sweet.*

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VARIETAL DIFFERENCES IN BARLEYS AND MALTS

VIII. CORRELATIONS BETWEEN ENZYMATIC ACTIVITIES AND MALT EXTRACT, MALTING LOSS, AND STEEPING TIME[1]

BY HENRY R. SALLANS[2] AND J. ANSEL ANDERSON[3]

Abstract

Significant negative inter-varietal correlation coefficients between steeping time and barley saccharifying (−0.706), malt saccharifying (−0.814), starch-liquefying (−0.954), autolytic diastatic (−0.937), and proteolytic (−0.822) activities are shown to be dependent on the same fundamental barley properties as similar positive coefficients between these activities and salt-soluble barley nitrogen. Barley and malt saccharifying and proteolytic activities are equally associated with both salt-soluble nitrogen and steeping time, but starch-liquefying and autolytic diastatic activities are more closely associated with the latter property. Significant inter-varietal correlation coefficients were obtained between liquefying, autolytic, and proteolytic activities and malt extract and malting loss. Partial correlation studies indicate that only the simple coefficient between malting loss and proteolytic activity (0.915) represents a real relation independent of salt-soluble nitrogen and steeping time.

With the exception of autolytic diastatic activity, highly significant intra-varietal correlation coefficients were obtained between malt extract and enzymatic activities. Partial coefficients, independent of total nitrogen, show that proteolytic, liquefying, and autolytic activities are associated with malt extract. Simple intra-varietal correlations of malting loss with barley saccharifying (0.671), malt saccharifying (0.702), and proteolytic (0.701) activities reflect mainly the effects of total nitrogen. Enzymatic activities are negatively correlated with steeping time within as well as between varieties, and partial correlation coefficients suggest a real relation between liquefying activity and steeping time.

Other papers in this series dealt with a number of barley, malt, and malting properties determined on the same set of 144 samples. The data obtained have made it possible to investigate the relations between a comparatively large number of pairs of properties. While all of these relations are not of equal importance or interest many of them are worth recording.

The development of enzymes is fundamental in the malting process and it seemed of value to study the interrelations of enzymatic activities with certain malt and malting properties. The present paper contains a discussion of the relations between barley saccharifying activity, saccharifying, starch-liquefying, autolytic diastatic, and proteolytic activities of malt on the one hand and malt extract, malting loss, and steeping time on the other.

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Data

The experimental data were obtained by analysis of 144 samples of barley and of the malts made from them. The samples represent 12 varieties grown at 12 widely separated experimental stations in Canada (1). Malting methods and summaries of the data on malt extract, malting loss, and steeping time were given in Part IV (4). Data on saccharifying activities of the barleys and malts were discussed in Part II (5) and Part III (2), those on proteolytic activity in Part VI (3), and those on starch-liquefying and autolytic diastatic activities in Part VII (6).

Correlat'on Studies

It was shown in previous papers (2, 3, 6) that significant inter-varietal relations existed between non-protein, salt-soluble protein, and total salt-soluble barley nitrogen and enzymatic activities, but that correlations of these activities with the other nitrogen fractions were not significant. In the case of intra-varietal relations the correlation coefficients between the enzymatic activities and total nitrogen, insoluble nitrogen, and alcohol-soluble nitrogen are highly significant, while those between these activities and the non-protein, salt-soluble protein, and total salt-soluble nitrogen represent a lesser degree of association. Thus, it appears that inter- and intra-varietal relations between enzymatic activities and other fundamental barley and malt properties are different in nature. For these reasons the inter- and intra-varietal relations are discussed in separate sections.

Inter-varietal Relations

The most interesting feature of the present study is that highly significant negative inter-varietal correlation coefficients were obtained between enzymatic activities and the time required to steep the barleys to a moisture content of 46%. In Part VII (6) it was shown that similar positive coefficients were obtained between the enzymatic activities and salt-soluble barley nitrogen. These simple correlation coefficients together with the multiple coefficients between the activities and salt-soluble nitrogen and steeping time are given in Table I. It appears that varieties that are high in salt-soluble nitrogen tend to yield malts of high enzymatic activity and those that require a long steeping time tend to yield malts of low activity.

The correlation coefficients in Table I were determined from variety means, and do not give information concerning the associations at any particular station. To determine whether they represent fundamental relations between enzymatic activities and steeping time, a more intensive study was undertaken. Regressions of the activities on steeping time were determined by stations and tested for homogeneity. From the results of these analyses, shown in Table II, it appears that the regressions do not differ significantly from station to station. Thus unit change in steeping time is accompanied by the same change in any given activity at all stations. This indicates that real relations exist between steeping time and the enzymatic activities.

Mean square residuals from the individual regressions of the activities on steeping time were tested against similar values for the regression on salt-soluble nitrogen (cf. 6, Table VI). It was found that the residual variances of barley saccharifying, malt saccharifying, and proteolytic activities did not differ significantly; but regressions of starch-liquefying and autolytic diastatic activities on steeping time accounted for significantly greater portions of the total variances of these activities than similar regressions on salt-soluble nitrogen. It may therefore be concluded that barley saccharifying, malt saccharifying, and proteolytic activities are equally associated with both salt-soluble nitrogen and steeping time; but that starch-liquefying and autolytic diastatic activities are more closely associated with steeping time than with salt-soluble nitrogen.

The multiple correlation coefficients between the enzymatic activities and salt-soluble nitrogen and steeping time were determined and are shown in the last column of Table I. Tests of residual variance (cf. 4, Table IX) showed

TABLE I

INTER-VARIETAL CORRELATION COEFFICIENTS AMONG ENZYMATIC ACTIVITIES (x) SALT-SOLUBLE BARLEY NITROGEN (n) AND STEEPING TIME (s)

x = enzymatic activity	Correlation coefficient		
	Simple r_{xn}	Simple r_{xs}	Multiple $R_{x.ns}$
Barley saccharifying	.739**	−.706**	.776**
Malt saccharifying	.727**	−.814**	.835**
Malt liquefying	.727**	−.954**	.955**
Autolytic diastatic	.750**	−.937**	.941**
Proteolytic	.871**	−.822**	.909**

NOTE: *In this and the following tables, ** indicates that the 1% level, and * that the 5% level of significance has been attained.*

that these multiple coefficients would not account for significantly greater portions of the variance than the higher of the two simple correlation coefficients. These considerations together with the fact that a significant simple correlation (−0.740**) exists between salt-soluble nitrogen and steeping time, indicate that the associations of steeping time and salt-soluble nitrogen with enzymatic activities are due to the same fundamental causes. It is difficult to interpret these data in terms of the physical and chemical properties of the barley. However, one appears forced to the conclusion that the soluble nitrogen fractions of the barley or some related factors influence the rate of absorption of water in the steep. Furthermore, it seems reasonable to assume that the relations between enzymatic activities and steeping time may be due to physical and chemical properties of the barleys that influence the rate of diffusion within the kernels.

It is evident from Table II that regressions of enzymatic activities on steeping time do not account for sufficiently high percentages of the variances of the activities to permit their prediction from steeping time. But, since both salt-soluble nitrogen and steeping time are related to all the activities, it is possible that these properties might be used in conjunction with a measure of potential extractives for the prediction of extract.

TABLE II

TEST OF HOMOGENEITY OF INTER-VARIETAL REGRESSION OF ENZYMATIC ACTIVITIES ON STEEPING TIME BY ANALYSIS OF RESIDUAL VARIANCE

Variance due to	Degrees of freedom	Mean square				
		Barley saccharifying	Malt saccharifying	Starch liquefying	Autolytic diastatic	Autolytic proteolytic
Differences among station regression coefficients	11	553.849	195.413	3,132.3	5,351.36	2,739.85
Deviations from individual station regressions	120	805.402	329.081	8,274.9	6,209.92	1,805.51
Percentage of intra-station variance accounted for by individual regressions		39.0	40.2	63.3	46.5	40.3

In Part VII (6) it was shown that the associations between enzymatic activities were due mainly to their common association with salt-soluble nitrogen. Since steeping time accounts for the same portions of the variance of the activities plus a greater portion in the case of liquefying and autolytic activities, partial correlation coefficients between the enzymatic activities, independent of both salt-soluble nitrogen and steeping time, were determined. These are shown in Table III. Only two are significant, namely, barley saccharifying—malt saccharifying and liquefying—autolytic.

It was demonstrated in Part VII (6) that determinations of barley and malt saccharifying activities were measures of β-amylase, and determinations of starch-liquefying and autolytic activities were measures of α-amylase. It is

TABLE III

INTER-VARIETAL PARTIAL CORRELATION COEFFICIENTS, BETWEEN ENZYMATIC ACTIVITIES, INDEPENDENT OF BOTH SALT-SOLUBLE NITROGEN AND STEEPING TIME

Enzymatic activity	Malt activities			
	Saccharifying	Liquefying	Autolytic diastatic	Proteolytic
Barley saccharifying	.785**	.351	.289	−.276
Malt saccharifying	—	.209	.417	−.344
Malt liquefying	—	—	.870**	.013
Malt autolytic diastatic	—	—	—	.028

therefore not surprising that the partial correlation studies indicate fundamental relations between these properties. The remaining coefficients all fall well below the 5% level of significance, and it appears that no direct association exists between these activities apart from their common association with certain barley properties. Salt-soluble nitrogen and steeping time reflect these physical and chemical properties, and it seems logical to use them as measures of potential enzymatic activity in studying the prediction of extract.

Inter-varietal correlation coefficients between enzymatic activities and malt extract and malting loss are shown in Table IV.

TABLE IV

INTER-VARIETAL SIMPLE CORRELATION COEFFICIENTS BETWEEN ENZYMATIC ACTIVITIES AND MALT PROPERTIES, AND PARTIAL COEFFICIENTS INDEPENDENT OF BOTH SALT-SOLUBLE NITROGEN AND STEEPING TIME

Enzymatic activity	Correlation coefficient			
	Malt extract		Malting loss	
	Simple	Partial	Simple	Partial
Barley saccharifying	.288	−.266	.436	−.642*
Malt saccharifying	.325	−.357	.465	−.675*
Malt liquefying	.592*	.136	.695*	.060
Malt autolytic diastatic	.653*	.362	.700*	−.005
Malt proteolytic	.644*	.448	.915**	.689*
Residual degrees of freedom	10	8	10	8

The simple coefficients in the first column indicate that associations exist between malt extract and liquefying, autolytic, and proteolytic activities; so that varieties which tend to be high in these activities tend to be high in extract also. The corresponding partial coefficients independent of salt-soluble nitrogen and steeping time are not significant and suggest that the physical and chemical factors responsible for the inter-varietal relations between the enzymatic activities also affect the extract yield. This hypothesis is supported by the fact that the simple correlation coefficient between malt extract and steeping time (−0.590*) was significant.

The simple correlation coefficients between enzymatic activities and malting loss are very similar to but higher than those for extract. The simple coefficient for proteolytic activity (0.915), computed from variety means, although quite high, does not, however, represent a particularly close relation in individual cases, for the regression of proteolytic activity on malting loss by stations accounted for only 46.6% of the total intra-station variance of proteolytic activity. However, the significant partial correlation independent of salt-soluble nitrogen and steeping time does suggest that proteolytic enzymes are an important factor controlling malting loss. It would appear that varieties well supplied with proteolytic enzymes grow vigorously, with

the result that malting loss tends to be high. The partial coefficients for liquefying and autolytic activities show that these have little effect on malting loss when the effects of salt-soluble nitrogen and steeping time are removed.

An apparent anomaly occurs in the case of partial coefficients, independent of salt-soluble nitrogen and steeping time, between malting loss and saccharifying activities. It is difficult to understand why these should be significant and negative. However, when the data were plotted, it was observed that for unit decrease in steeping time the saccharifying activities did not increase as much as would be expected from the increase in malting loss. This effect was particularly marked with the varieties Charlottetown 80, Hannchen, Victory, Regal, and Velvet, and is sufficient to account for the negative partial coefficients. Since the simple coefficients for saccharifying activities and malting loss are not significant and the partials are negative, it must be concluded that these activities are not related to malting loss. This was to be expected, because it was shown in Part VII (6) that the liquefying or α-amylase activity was the most important factor governing autolysis in the malts and that saccharifying or β-amylase activity had no significant effect on this reaction.

Intra-varietal Relations

The intra-varietal relations between enzymatic activities and malting properties are shown in Table V. In view of the significant correlation of enzymatic activities with total nitrogen (6) and with certain of these malting properties, partial coefficients were determined to show whether relations exist independent of a common association with total nitrogen.

TABLE V

INTRA-VARIETAL SIMPLE CORRELATION COEFFICIENTS, AND PARTIAL COEFFICIENTS INDEPENDENT OF BARLEY NITROGEN, BETWEEN ENZYMATIC ACTIVITIES AND MALT PROPERTIES

Enzymatic activity	Correlation coefficient					
	Malt extract		Malting loss		Steeping time	
	Simple	Partial	Simple	Partial	Simple	Partial
Barley saccharifying	−.918**	.248	.671*	−.039	−.626*	−.503
Malt saccharifying	−.931**	−.143	.702*	.178	−.661*	−.228
Malt liquefying	−.728**	−.547	.281	−.282	−.881**	−.801**
Malt autolytic diastatic	.084	−.619*	.189	.542	−.269	−.593
Malt proteolytic	−.949**	−.874**	.701*	.290	−.704*	−.400
Residual degrees of freedom	10	9	10	9	10	9

Simple correlation coefficients between the enzymatic activities and malt extract indicate that within varieties, with the exception of autolytic activity, environmental conditions which increase enzymatic activities tend to decrease extract. The partial coefficients for proteolytic and autolytic diastatic activities are significant and that for liquefying sufficiently high to suggest that

these relations between extract and the activities are not entirely explained by association of both factors with total nitrogen. However, it appears that in general an increase in total nitrogen within varieties is accompanied by an increase in enzymatic activity and a decrease in extract yield.

The relation between proteolytic activity and extract is particularly interesting, since the high partial coefficient suggests that quite a close association exists between extract and proteolytic activity apart from a common association with total nitrogen. The multiple correlation coefficient for extract and proteolytic activity and total nitrogen proved to be 0.990, which is significantly higher than either of the simple coefficients. It appears that the relation between nitrogen and extract, which is negative, reflects a positive relation between the carbohydrates and extract; and the addition of proteolytic activity in the relation gives the added effect of the soluble nitrogen on malt extract.

There seems to be some intra-varietal association between malting loss and saccharifying and proteolytic activities. The partials, however, suggest that the simple correlation coefficients reflect mainly the correlations between each variable and total nitrogen.

Steeping time and enzymatic activities are negatively associated within as well as between varieties. The partial correlation coefficients show also that starch-liquefying activity is highly correlated with steeping time independent of the effect of total nitrogen. The inter- and intra-varietal associations between liquefying activity and steeping time appear to be so close that one is forced to conclude that some causal relation must exist between them. It appears that the longer steeping time required to reach a given moisture content reflects a greater resistance to absorption of water and possibly also to movement of water within the kernel. This might well influence both the rate of activation of enzymes and their rate of reaction with substrates.

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QUALITATIVE STUDIES OF SOIL MICRO-ORGANISMS

III. INFLUENCE OF PLANT GROWTH ON THE CHARACTER OF THE BACTERIAL FLORA[1]

By A. G. Lochhead[2]

Abstract

Comparative studies of the relative incidence of bacterial types occurring in the rhizosphere of different plants and in control soils indicated that the qualitative nature of the soil microflora is markedly influenced by the growing plant. In the rhizosphere Gram-negative rods are proportionately increased while Gram-positive rods, coccoid rods, and spore-forming types are relatively less abundant.

The majority of bacteria isolated from soil by non-selective plating are forms included in the family Proactinomycetaceae (Jensen's classification). Of these by far the largest group consists of members of the genus *Corynebacterium*. In the rhizosphere proactinomycetes as a whole are relatively less abundant, with the *Corynebacterium* (non-motile) group likewise depressed. However, closely related motile forms classed as *Mycoplana* are preferentially stimulated.

In the rhizosphere the bacteria show definitely greater physiological activity than in soil distant from the plant. Not only is there a notably greater proportion of motile forms, and a pronounced increase in the incidence of chromogenic types, but also a higher incidence of liquefying bacteria and of those able to affect glucose.

A comparison of the rhizosphere of certain plant varieties resistant and susceptible respectively to soil-borne disease showed differences of a qualitative nature in the bacterial flora suggestive of a greater "rhizosphere effect" in the case of the susceptible varieties studied. Results point to the possibility that resistance may be associated with a selective action of root excretions on the saprophytic soil microflora.

Introduction

In a previous paper of this series (16), following a general introduction (9), a report was made of investigations carried out on a non-selective basis of the qualitative nature and relative incidence of the bacterial types occurring in soils differing in fertility. On the basis of morphology in soil extract semi-solid medium, in which the organisms were considered to correspond more closely to their form in soil than when cultivated on more "synthetic" substrates, the bacteria were classified into eight groups. In spite of unequal productivity the soils showed no outstanding differences in the relative abundance of the various morphological groups, while grouping on the basis of physiological activity likewise showed a surprising degree of uniformity in the soils studied. A brief summary of the characters of the organisms found is given in Table I, recalculated from previous data for comparison with findings as to the effect of plant growth presented below.

The results suggested that the character of the *autochthonous* (indigenous) soil microflora is relatively uniform in soil of definite type, and comparatively

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little affected by normal field application of fertilizers resulting in greatly altered crop-producing capacity. The predominant soil bacteria appeared relatively inactive in single culture, with considerable divergence in biochemical action shown by apparently closely related forms. The findings suggested that the bacterial flora of soil is unstable physiologically, with considerable adaptability, and that the functions of the different types are exercised most fully only under conditions of association.

The object of the present investigation was to study the effect of the growing plant upon the nature of the soil bacteria, and compare the characters of bacteria in the rhizosphere with those of organisms away from the zone of influence of the plant.

The Rhizosphere—Qualitative Aspects

Some 35 years ago Hiltner (1) observed that certain micro-organisms showed increased activity close to the roots of cultivated plants and called this zone influenced by root excretions the "rhizosphere". Since then various other investigators have confirmed his findings, and shown that soil in the vicinity of the roots supports usually noticeably higher numbers of micro-organisms than soil more distant from the plant. Purely quantitative considerations, however, need not concern us here.

Our information as to the effect of plant growth on the qualitative nature of soil bacteria is meagre, and confined largely to data on the numbers of organisms appearing on selective media chosen to favour various groups. Thus Starkey (13, 14) found that the development of higher plants exerted greater influences upon some groups of organisms than upon others. Whereas the proportionate increases in nitrogen-fixing organisms, actinomycetes, and filamentous fungi were slight, organisms of the *B. radiobacter* group were preferentially stimulated in the rhizosphere. It was found, however, that different plants exerted different degrees of influence upon soil micro-organisms.

Krassilnikov (6) reported that *Azotobacter* was unable to grow in the rhizosphere of wheat and was severely repressed in that of maize, and attributes this to the toxic effect of root secretions. Similarly *B. mycoides*, *B. megatherium*, and yeasts of the type of *S. cerevisiae* were unable to grow in the rhizosphere of the same plants. On the other hand, small non-sporing bacteria of the types of *B. denitrificans* and *B. fluorescens* were found to multiply intensively in the rhizosphere, presumably under the stimulation of organic root excretions.

Evidence of a selective action exerted by the root system on soil bacteria was obtained by Krassilnikov, Kriss, and Litvinov (7), who reported striking increases in cellulose-decomposing organisms and of small non-spore-forming rods growing poorly under laboratory conditions. The function of this latter group of bacteria, which are characterized by ability to reduce nitrates to nitrites, is unknown. The same authors in a later paper (8) found the predominant organisms in the rhizosphere to consist of non-sporing rods with

well pronounced ammonifying capacity, and of mycobacteria. Spore-formers comprised an insignificant proportion of the rhizosphere population, though some rise was apparent towards the end of the vegetation period of the plants.

Further information as to predominant bacterial types developing about roots of growing plants has been given by Starkey (15) from microscopic observations of the rhizosphere. Employing the buried slide technique, he observed that the bacteria occurring in greatest abundance about root hairs, fungus filaments, and decomposing organic matter were small, coccoid cells. Longer rods were detected, but spore-formers were seldom encountered.

Studying the effect of organic soil amendments on the microflora of wheat roots, Thom, Clark, Fierke, and Fellows (17) found corynebacteria to be the most numerous types when either chicken manure or chopped green alfalfa was added to soil, though certain differences in physiological properties of the predominant organisms were observed under the two conditions. The observations were concerned with the influence of soil treatment rather than with the effect of the plant in modifying the soil microflora.

Experimental

The present report embraces qualitative studies of the bacterial flora of the rhizosphere of red clover, mangels, oats, tobacco, corn, and flax to note changes exerted by the various crops on the relative incidence of types present in control soils. In addition, comparative studies were made of the rhizosphere of certain varieties of tobacco and flax resistant and susceptible respectively to soil-borne fungous diseases.

For the analysis of field samples, blocks of soil about the plants were carefully dug up and brought to the laboratory. After removal of above-ground portions of the plants, the soil was gently broken to release the root system, which was added to sterile water together with adherent soil after gentle shaking to remove superfluous quantities. After thorough shaking suitable dilutions were prepared from the original flask for plating. The roots were later removed, contents of flask measured and evaporated to dryness to permit of the estimation of plate counts of rhizosphere soil on a dry weight basis. Controls consisted of corresponding composite soil samples distant from the plant; in the case of different plant varieties, e.g., tobacco, the rhizospheres of which were being compared, midway between rows. In the case of the greenhouse samples, plants were carefully removed from the pots and similarly treated. Controls in this case consisted of the remainder of the soil in the pot after thorough mixing.

Examination of rhizosphere and control soil was made on a quanti-qualitative basis following general procedures previously described (16). This involved a study of colonies growing on soil extract agar prepared according to Löhnis (10) without added energy material, and chosen as being as non-selective as possible, and hence most desirable for primary isolation purposes. While a "richer" medium would allow better colony development of many forms, this was considered a distinct disadvantage in view of the antagonisms

possible in plate cultures leading to the suppression of other forms which might otherwise appear, even as small colonies.

From representative plates, 60 to 100 colonies were picked, representing all on a plate or a sector, and stab cultures made to soil extract semi-solid medium (containing 0.02% K_2HPO_4, 0.01% yeast extract and 0.3% agar). The use of this medium assured the survival of 93% of the transfers, many from pin-point colonies. Obvious actinomycetes were not included. The present report is based on data from a study of 1,191 cultures.

Morphological observations of predominant soil types had indicated the presence of a large proportion of pleomorphic organisms, which in the majority of cases showed affinity to the corynebacteria. It was also observed that whereas on the richer artificial media pleomorphism was quite pronounced, little or none was observed in sterilized soil, or in soil extract semi-solid medium. Classification of the strains isolated, therefore, was made (i) on the basis of the forms occurring in soil extract semi-solid medium and considered to represent more closely the morphology of the organisms in soil, and (ii) on the basis of additional observations on a variety of richer artificial media to differentiate forms with a view to noting more closely their taxonomic relationship. For this purpose nutrient agar and yeast peptone agar as employed by Topping (19, 20) in her soil flora studies were used. Tests for motility, staining reactions, and biochemical properties were included to aid in grouping.

Comparison of Rhizosphere and Control Soils

In Table II is presented a summary of morphological groups as observed in soil extract semi-solid medium, in addition to certain physiological groups, derived from a study of individual cultures from the rhizosphere of various cultivated plants. In contrast to Table I, which indicates little or no difference due to fertilizer treatment, Table II reveals certain striking effects exerted by the growing plant on the bacterial groups in the soil.

While different plants show variations, both in total numbers and in the incidence of different microbial groups, yet there is noted an unmistakable selective action characteristic of the rhizosphere of all plants studied. In all cases Gram-negative short rods are proportionately increased in the rhizosphere as compared with the control soil. On the other hand Gram-positive short rods, coccoid rods, and spore-forming bacteria are relatively less numerous in the rhizosphere than in soil more distant from the plant. In the case of Gram-variable rods, *Bact. globiforme* group, cocci, and long non-sporing rods, no definite rhizosphere effect is noted suggestive of group stimulation or depression due to plant growth.

From the standpoint of bacterial physiology, the findings indicate a more active bacterial flora in the rhizosphere than in the soil beyond the zone of influence of the plant. In the rhizosphere is found a higher percentage of forms developing well on nutrient agar, of liquefying types, and of those causing acid or alkaline reactions in dextrose. A noticeably greater percentage

TABLE I

COMPARISON OF BACTERIAL GROUPS IN SOILS OF DIFFERENT FERTILITY

	Soil N (no fertilizer)	Soil X (farmyard manure)	Soil Y (mineral fertilizer)
Relative crop producing capacity			
Timothy	55.9	100.0	85.1
Mangels	8.9	100.0	86.5
Morphological groups (soil extract)	%	%	%
Short rods, Gram-pos.	29.6	30.5	24.5
Short rods, Gram-neg.	30.5	35.2	42.1
Short rods, Gram-var.	7.0	10.8	10.3
Bact. globiforme group	11.2	7.5	8.3
Coccoid rods, Gram-pos.	8.4	5.6	3.9
Cocci	5.6	3.3	2.4
Long rods, non-sporing	3.3	3.8	4.4
Spore-formers	4.2	3.2	3.9
Physiological groups	%	%	%
Growth on N. A. very slight or absent	53.5	58.7	66.1
Gelatin liquefaction	28.6	27.7	29.4
Nitrate reduction	40.4	43.7	30.4
Acid reaction in dextrose 1%	42.7	38.5	43.6
Alkaline reaction in dextrose 1%	21.6	16.9	15.7

of motile organisms is present in the rhizosphere, while the incidence of chromogenic types shows a very pronounced increase over that in the control soils.

The more detailed comparison of the tobacco, corn, and flax soils, based on more extended observation to note specially pleomorphic forms, is summarized in Table III. Further points of interest are brought out in the relative incidence of certain broad generic groups as affected by plant growth. Non-sporing, non-pleomorphic, rod-shaped bacteria are proportionately increased in the rhizosphere, particularly Gram-negative forms. On the other hand, the broad group of pleomorphic organisms, comprising well over half the forms in the control soils, are relatively less abundant.

Investigations of such workers as Jensen (2–4), Ørskov (11, 12), Krassilnikov (5), Topping (19), and Umbreit (21) have called attention to the occurrence in soil of appreciable numbers of organisms belonging to non-sporing genera of the order Actinomycetales, forms showing characteristically the properties of bacteria, though usually with a considerable degree of pleomorphism and in some cases with a tendency towards formation of slight mycelium. The taxonomy of the order is at present confused and the nomenclature for what are apparently similar groups most varied. The classification of soil forms is hindered not only by lack of sufficient study, but also because many soil species do not appear to fit into schemes of classification based largely on the more "classical" parasitic forms.

TABLE II

INCIDENCE OF BACTERIAL GROUPS IN RHIZOSPHERE AND CONTROL SOILS (A)

Classification	Rotation plots (N)				Tobacco soil			Corn soil		Flax (pot expt.)		
	Control	Rhizosphere			Control	Rhizosphere		Control	Rhizo.	Control	Rhizosphere	
		Clover	Mangels	Oats		RH. 211	CH. 38				Bison	Novelty
Plate count (millions)	48.6	254.8	108.2	118.1	94.7	269.2	505.4	28.2	389.2	98.3	439.0	2751.3
Morphology, Soil ex. semi-solid	%	%	%	%	%	%	%	%	%	%	%	%
Short rods, Gram-pos.	19.7	8.7	10.8	13.6	23.3	20.0	16.1	23.1	22.2	7.7	6.1	1.4
Short rods, Gram-neg.	30.3	55.1	43.1	45.5	13.3	43.3	45.2	26.9	51.1	15.4	36.4	59.1
Short rods, Gram-var.	16.7	15.9	15.4	19.7	11.7	6.7	17.7	5.8	6.7	7.7	7.1	14.1
Bact. globiforme group	7.6	1.5	10.8	1.5	13.3	10.0	16.1	7.7	4.4	1.9	16.2	1.4
Coccoid rods, Gram-pos.	13.6	5.8	10.8	10.6	18.3	10.0	4.8	21.2	13.3	53.8	32.3	16.9
Cocci	1.5	1.5	3.1	3.0	1.7	3.3	0.0	0.0	0.0	7.7	0.0	1.4
Long rods, non-sporing	4.6	8.7	6.2	3.0	6.7	5.0	0.0	5.8	2.2	0.0	1.0	5.6
Spore-formers	6.1	2.9	0.0	3.0	10.0	1.7	0.0	9.6	0.0	5.8	1.0	0.0
Physiology												
Good growth, nutrient agar	33.3	56.5	61.5	45.5	35.0	75.0	66.1	48.1	57.7	36.5	78.8	85.9
Chromogenic types	12.1	29.0	29.2	21.2	1.7	26.7	29.0	3.8	33.3	7.7	29.3	25.4
Motile forms	—	—	—	—	13.3	31.7	25.8	13.5	24.4	26.9	36.4	64.8
Liquefying (1 wk.)	—	—	—	—	15.0	16.7	41.9	23.1	24.4	42.3	54.6	70.4
Inhibited, 1% dextrose	42.4	27.5	20.0	22.7	30.0	25.0	25.8	11.5	8.9	5.8	1.0	0.0
Acid reaction, dextrose	30.4	42.1	53.8	43.9	36.7	38.3	48.4	56.7	57.8	50.0	67.7	81.7
Alk. reaction, dextrose	6.1	15.9	16.9	21.2	16.7	21.7	17.7	9.6	17.8	3.9	9.1	12.7

TABLE III

INCIDENCE OF BACTERIAL GROUPS IN RHIZOSPHERE AND CONTROL SOILS (B)

Classification	Tobacco soil			Corn soil		Flax (pot expt.)		
	Control	Rhizosphere		Control	Rhizo.	Control	Rhizosphere	
		RH. 211	CH. 38				Bison	Novelty
Plate count (millions)	94.7	269.2	505.4	28.2	389.2	98.3	439.9	2751.3
Non-pleomorphic	%	%	%	%	%	%	%	%
Cocci	1.7	1.7	0.0	0.0	0.0	1.9	0.0	0.0
Rods, spore-forming	10.0	1.7	0.0	9.6	0.0	5.8	1.0	0.0
Rods, non-sporing, total	21.6	41.7	40.3	28.8	44.4	11.5	24.2	54.9
Rods, non-sporing, Gram-pos.	18.3	20.0	20.9	21.2	24.4	5.8	10.1	18.3
Rods, non-sporing, Gram-neg.	3.3	21.7	19.4	7.5	20.0	5.8	14.1	36.6
Rods, non-sporing (pct. Gram-pos.)	(84.6)	(45.8)	(52.0)	(73.3)	(55.0)	(50.0)	(41.7)	(33.3)
Rods, non-sporing (pct. motile)	(38.5)	(48.0)	(41.7)	(6.7)	(30.0)	(33.3)	(50.0)	(66.7)
Rods, non-sporing (pct. chromogenic)	(0.0)	(32.0)	(28.0)	(13.3)	(53.0)	(0.0)	(41.7)	(26.2)
Pleomorphic								
Total (Proactinomycetaceae)	65.0	55.0	58.1	53.9	48.9	80.7	74.5	45.1
Acid-fast, non-motile, Gram-pos. (*Mycobacterium*)	8.3	0.0	1.6	1.9	2.2	3.8	0.0	0.0
Non-acid-fast, non-motile (*Corynebacterium*)								
Total	55.0	45.0	45.2	42.3	35.6	57.7	50.5	16.9
Gram-pos.	43.3	33.3	38.7	40.4	24.4	44.2	42.4	8.5
Gram-neg.	11.7	11.7	6.5	1.9	11.1	13.5	8.1	8.5
Non-acid-fast, motile (*Mycoplana*)								
Total	1.7	10.0	11.3	9.6	11.1	19.2	24.2	28.2
Gram-pos.	1.7	1.7	6.5	9.6	8.9	13.5	13.1	5.6
Gram-neg.	0.0	8.3	4.8	0.0	2.2	5.8	11.1	22.6
Non-acid fast (pct. Gram-pos.)	(81.9)	(63.7)	(80.0)	(96.3)	(71.4)	(75.0)	(74.3)	(35.5)
Non-acid-fast (pct. motile)	(2.9)	(18.2)	(20.0)	(18.4)	(23.8)	(25.0)	(32.4)	(61.3)
Non-acid-fast (pct. chromogenic)	(2.9)	(24.2)	(31.4)	(0.0)	(19.0)	(10.0)	(24.3)	(22.6)
Non-acid-fast (pct. liquefy.)	(14.7)	(24.2)	(54.3)	(22.2)	(23.8)	(52.5)	(56.8)	(54.8)
Non-acid-fast (pct. reduce NO_3)	(47.1)	(30.3)	(42.8)	(22.2)	(18.0)	(50.0)	(14.9)	(41.9)

Pleomorphic types considered to fall within the family Proactinomycetaceae following Jensen's (2) classification of the Actinomycetales, were found to comprise a large proportion of the bacteria in the control soils. It was found that clear-cut distinction of strains on the basis of such properties as slight mycelium formation, acid fastness, or reaction to the Gram stain is not entirely satisfactory in the separation of forms occurring in soil. Jensen (2) has already suggested that the transition from *Proactinomyces*, the criterion for which genus is the formation of initial mycelium, to *Mycobacterium* and *Corynebacterium* is very gradual, while Topping (19), Ørskov (12), and Umbreit (21) have also stressed the difficulty of definite distinction between these groups on the basis of slight mycelium formation. In the soils studied, mycelium formation was observed with relatively few strains and in the absence of other pronounced features distinguishing this group, they were classified, for the present purpose, with the acid-fast group (mycobacteria) or the non-acid-fast group (corynebacteria).

Non-acid-fast forms comprised the overwhelming majority of the proactinomycetes in the soils examined. These organisms exhibited characteristic "snapping" division in the rod stage and showed a tendency, particularly on richer artificial media, towards the production of irregular, swollen forms, often slightly branched or showing formation of buds or "sprouts". In older cultures, and more especially in soil extract media, the organisms appeared more predominantly as coccoids, arising from a process of fragmentation. Comparison of these forms, which showed closest affinity to the genus *Corynebacterium*, indicated that many apparently closely related strains differed in their reaction to the Gram stain and with respect to motility. Since the generic term *Corynebacterium* is usually reserved for non-acid-fast, non-motile, Gram-positive forms, the taxonomy of motile or Gram-negative forms may well be a matter of dispute. Some provision appears necessary for motile soil forms related to corynebacteria, the presence of which has been also stressed by Topping (19) and Ørskov (12). For the present purpose, non-motile forms are classified as *Corynebacterium*, with recognition of both Gram-positive and Gram-negative types, while motile forms are considered to show closest affinity to the genus *Mycoplana* Gray and Thornton, to include likewise both Gram-positive and Gram-negative forms. Whether this separation of motile from non-motile forms is preferable to the inclusion of both forms in the genus *Corynebacterium* will doubtless await further investigation, though some support of the latter alternative is given by our finding of occasional motile strains of the *Bacterium globiforme* group. The characteristic *Bact. globiforme* is now believed by us to represent a special group of the corynebacteria with distinctive cultural and physiological properties. In the present comparison of control soils and rhizosphere, where formal classification and identification of species were regarded as less important than the grouping of organisms according to certain morphological and physiological characters, it was found of advantage to consider motility and Gram reaction in differentiating between strains.

As noted from Table III the relative incidence of proactinomycetes as a whole is lower in the rhizosphere than in the control soils. Within the broad group, however, a selective action by the plant is apparent. The largest group in the control soils consists of non-acid-fast, non-motile forms regarded as corynebacteria. This group appears to correspond to organisms found by Jensen (3) to comprise an important group of organisms in Australian soils, and are doubtless to be included with the "mycobacteria" reported by Krassilnikov (5) to be abundant in Russian soils. This group showed a relative depression in the rhizosphere. On the other hand, motile forms, provisionally grouped as *Mycoplana*, appeared to be preferentially stimulated in the rhizosphere of all plants studied.

With pleomorphic as well as non-pleomorphic bacteria, Gram-positive forms were relatively less numerous in the rhizosphere than in controls, while chromogenic forms were conspicuously more abundant in the rhizosphere. Proactinomycetes with liquefying capacity were rather more abundant in the rhizosphere, though nitrate-reducing forms appeared to be slightly less numerous relatively as compared with the control soils.

Rhizosphere of Resistant and Susceptible Plants

In comparative quantitative studies of the rhizosphere of certain varieties resistant and susceptible respectively to soil-borne pathogens, Timonin (18), working in this laboratory, noted higher numbers of bacteria, and to a less extent, of fungi, in the case of the susceptible than of resistant varieties. This was observed in both greenhouse and field tests with flax susceptible to wilt (*Fusarium lini*), and with tobacco susceptible to black root rot (*Thielaviopsis basicola*). With actinomycetes no difference was observed. Since the tests were made with uninfected plants, the differences observed were believed to be due to inherent differences in physiological function, making in the case of susceptible plants, conditions somewhat more favourable for general bacterial development.

Qualitative surveys of the bacterial flora of the rhizosphere which included comparisons of resistant (Bison) and susceptible (Novelty) flax, and resistant (RH. 211) and susceptible (CH. 38) tobacco, are partly summarized in Table IV. Morphological classification according to forms developing in soil extract semi-solid medium showed differences in the relative incidence of three groups of organisms. Gram-negative short rods were relatively more abundant, and coccoid rods and spore-formers less abundant, in the rhizosphere of the susceptible than of the resistant varieties of plants. As noted above (Table II) the same trend was observed with respect to rhizosphere and control soil. These findings, taken in conjunction with the quantitative data, suggest that the susceptible plants studied exerted a greater "rhizosphere effect" than did corresponding resistant varieties.

The more detailed differentiation showed few significant differences. Non-motile, non-pleomorphic short rods, and motile, pleomorphic rods (*Mycoplana?*) showed a slightly higher incidence in the rhizosphere of susceptible plants.

TABLE IV

BACTERIA IN RHIZOSPHERE OF RESISTANT AND SUSCEPTIBLE PLANTS

Classification	Flax (field)		Flax (pots)		Tobacco (field)	
	Bison (R)	Novelty (S)	Bison (R)	Novelty (S)	RH. 211 (R)	CH. 38 (S)
Plate count (millions)	13.1	18.7	439.9	2751.3	269.2	305.4
Morphology—Soil ex. semi-solid	%	%	%	%	%	%
Gram-neg. short rods	32.2	44.4	36.4	59.1	43.3	45.2
Coccoid rods, Gram-pos.	10.2	6.3	32.3	16.9	10.0	4.8
Spore-formers	16.9	4.8	1.0	0.0	1.7	0.0
Detailed differentiation (non-sporing types)						
Non-pleomorphic						
Cocci	1.7	0.0	0.0	0.0	1.7	0.0
Rods, non-sporing, total	22.1	23.9	24.2	54.9	41.7	40.3
Rods, non-sporing, Gram-pos.	5.1	4.8	10.1	18.3	20.0	20.9
Rods, non-sporing, Gram-neg.	17.1	19.1	14.1	36.6	21.7	19.5
Rods, non-sporing, motile	6.8	6.3	12.1	36.6	20.0	16.1
Rods, non-sporing, non-motile	15.3	17.6	12.1	18.3	21.7	24.2
Pleomorphic						
Acid-fast, non-motile, Gram-pos.	1.7	7.9	0.0	0.0	0.0	1.6
Non-acid-fast, non-motile, total	39.0	46.0	50.5	16.9	45.0	45.2
Non-acid-fast, non-motile, Gram-pos	23.4	28.6	42.4	8.5	33.3	38.7
Non-acid-fast, non-motile, Gram-neg.	13.6	17.4	8.1	8.5	11.7	6.5
Non-acid-fast, motile, total	13.5	14.3	24.2	28.2	10.0	11.3
Non-acid-fast, motile, Gram-pos.	8.4	9.5	13.1	5.6	1.7	6.5
Non-acid-fast, motile, Gram-neg.	5.0	4.8	11.1	22.6	8.3	4.8
Physiological						
Motile organisms	28.8	30.1	36.4	64.5	31.7	25.8
Chromogenic forms	5.1	6.3	29.3	25.4	26.7	29.0
Liquefying	15.3	15.9	54.6	70.4	16.7	41.9
Acid reaction, dextrose	45.7	46.0	67.7	81.7	38.3	48.4
Nitrate-reducing	30.5	31.7	21.2	49.3	35.0	41.9

Physiological tests pointed, if anything, to a somewhat more active microflora in the rhizosphere of susceptible plants.

Although these preliminary findings by no means prove, yet they suggest the possibility, that resistance to certain disease may be linked up with a selective action of root excretions upon the saprophytic soil microflora, thus favouring types which may be more, and in other cases less, antagonistic (directly or indirectly) towards pathogenic organisms.

Conclusion

The results as a whole emphasize the importance of enlarging our knowledge of the qualitative nature of the soil micro-organisms and particularly those types the functions of which are still unknown, but which appear to comprise a large proportion of the micro-population of arable soils. Up to the present

the study of processes, and incidentally that of specialized groups of organisms concerned in these processes, has received more attention than the objective study of soil organisms on a non-selective basis. It is believed, however, that more study from the qualitative side is essential to help complete our knowledge of the types indigenous in soil and the changes in the trend of population in the rhizosphere. It is here where not only the main interaction occurs between soil micro-organisms and the growing plant, but where the action of various soil-borne pathogens or toxic factors occurs to complicate and accentuate asociative or antagonistic influences.

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CROSSING AND SELFING STUDIES WITH PHYSIOLOGIC RACES OF OAT STEM RUST[1]

By Thorvaldur Johnson[2] and Margaret Newton[3]

Abstract

Evidence has been obtained through crossing and selfing studies that some physiologic races of oat stem rust contain both homozygous and heterozygous lines, the latter when selfed tending to produce races more virulent than the selfed race. In crosses between races, the less virulent characteristics are generally dominant so that the hybrid race is often identical with the less virulent of the two parent races. Selfing studies and crosses therefore indicate that the more virulent characteristics of oat stem rust are recessive traits. Reciprocal crosses between physiologic races suggest that the cytoplasm, as well as the nucleus, contributes to the inheritance of certain pathogenic characters.

In crosses between races of normal (red) and orange uredial colour, the red colour has invariably proved dominant, the progeny being indistinguishable in colour from the normal parent.

Two selfing studies of Race 3 carried out with the same telial material at an interval of more than four years have provided evidence of a genetic change presumably brought about through the ageing of the teliospores. In the first selfing, no abnormalities were observed in the infections on the barberry. In the second selfing, about half of the infections developed pycnia and pycnial nectar, while the remainder appeared as small, round, necrotic areas showing no indication of even rudimentary pycnial formation.

Introduction

One of the difficulties encountered in inheritance studies with physiologic races of stem rust is the scarcity of characters whose inheritance can be studied. The urediospores of the different races are, for all practical purposes, identical morphologically. An inheritance study based on morphological characteristics is therefore out of the question. While differences in spore colour exist among the races collected in nature, these are, in general, so slight as to be almost imperceptible. It is only when races of decidedly abnormal spore colour arise, such as the orange races described in the present paper, that the inheritance of spore colour can be studied. Such colour variants have, however, not yet been discovered in the field and are but rarely encountered in the laboratory.

In the absence of definite morphological differences between races, inheritance studies must perforce be limited largely to pathogenic characteristics which find expression in the so-called "infection types" on seedling leaves of cereals. The fact that an infection type of a physiologic race is relatively constant on a given host variety grown under given conditions makes it permissible to regard it as a character of the rust. Its inheritance may then be investigated in crosses and selfing studies. A race of oat stem rust produc-

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ing a 2 infection type on the oat variety, White Tartar, may be crossed with another race producing a 4 infection type, the object being to discover the infection type of the hybrid race on White Tartar. Other infection types produced on other host varieties may be studied in a similar way. In selfing studies with races, it is possible to discover whether they are homozygous or heterozygous for each of the various infection types they produce on the differential oat varieties.

The work reported in the present paper was undertaken with the object of studying the breeding behaviour of known physiologic races of *Puccinia graminis Avenae* Erikss. and Henn., first, through the selfing of each race and, second, through crosses between the different races. The selfing studies were expected to furnish an indication of what might happen in nature when races infect barberries and spread from them. The crossing studies were expected to yield information on the pathogenic characters of the F_1 hybrid races in comparison with those of the parent races. If this program could have been carried through to completion for all the races known at present, it might have been possible to predict to a certain extent the pathogenic characteristics of new races that might be expected to arise, in nature, as new combinations on the barberry.

During the course of this work, however, certain obstacles have intervened to prevent its completion, particularly the sparseness of teliospore formation in races 1,2, and 5, and the difficulty of inducing greenhouse-formed teliospores of certain races to germinate. Nevertheless, selfing studies have been conducted, though sometimes on a limited scale, with seven of the twelve races known at the present time, and crosses have been made between several physiologic races. Although the work is not yet by any means complete, it is the opinion of the writers that sufficient evidence has been secured to warrant some generalization on the inheritance of certain of the pathogenic characteristics of oat stem rust.

Selfing Studies

The procedure followed and the technique employed in the selfing studies here reported have been described in detail elsewhere (7). Briefly stated, it consisted in (i) developing teliospores of pure cultures of the various physiologic races, (ii) inducing the teliospores to germinate, (iii) infecting the barberry with the sporidia, (iv) infecting oat seedlings with the aeciospores, and (v) identifying the physiologic races present in the resulting uredial cultures.

To gain an idea of the numerical distribution of physiologic races in the progeny of any selfing, aecial cups were picked off singly, at random, from numerous aecial pustules, crushed, and transferred individually to seedling leaves of a susceptible oat variety. In each selfing study this method was usually supplemented by one or two mass transfers of aecia from a large number of aecial pustules, the object of which was to ascertain whether all the races present on the barberry were represented in the random collection of aecia. The identification of the physiologic races was accomplished by

means of the three differential varieties White Tartar, Richland, and Joanette Strain, originally selected by Stakman, Levine, and Bailey (10) and by Bailey (1). On these varieties the different physiologic races produce characteristic infection types, designated by Bailey as 1, 2, 3, 4, and x (Table I). These infection types may be regarded as an expression of either the pathogenicity of the rust or the reaction of the host plant. Viewed in terms of host reaction, types 1 and 2 are regarded as indicating resistance, owing to the smallness of their pustules and the presence of necrosis and chlorosis, while type 4 with its large pustules is regarded as indicating susceptibility. In the text, types 1 and 2 are frequently referred to as small pustules and type 4 as large pustules to avoid constant mention of these infection types by numerals. The infection types chiefly dealt with in this paper—types 1, 2, 4, and x—are shown in Plate I, Fig. 9.

TABLE I

MEAN INFECTION TYPES PRODUCED BY PHYSIOLOGIC RACES OF *P. graminis Avenae* ON SEEDLING LEAVES OF OAT DIFFERENTIAL VARIETIES

Physiologic race	White Tartar	Richland	Joanette Strain
1	2+	1	1±
2	2++	1+	4
3	4−	1	1−
4	4	4+	1
5	2++	1++	x±
6	4+	4+	4+
7	4	2=	4−
8	2	4	3++
9	2±	x+	4
10	2	4	x+
10*a**	2+	4	1±
11	2	4	1=
12**	4=	1++	x+

* *Race 10a resembles the Race 11 described by Dr. K. Hassebrauk (Arb. biol. Reichs. 22 : 479-482. 1938) but as it occasionally produces an x type on Joanette Strain it is regarded as a variant of Race 10.*

** *The writers are indebted to Dr. E. C. Stakman and Dr. M. N. Levine for assigning a number to this race. They originally numbered it 11, but owing to the fact that Dr. Hassebrauk in the meantime had given this number to another race they suggested that the number of the new race be changed to 12.*

THE SELFING OF RACES COLLECTED IN THE FIELD

The earliest record of an artificial selfing of a known race of oat stem rust is a selfing of Race 8 reported by Gordon and Welsh (3). In a study of cultures arising from 16 separate aecial cups and four mass transfers of aecia, Race 8 occurred 15 times, Race 6 occurred seven times, and Race 7 once.

The selfing studies carried out by the present writers with races collected in nature are summarized in Table II. While some of these studies were

TABLE II

THE SELFING OF RACES OF *P. graminis Avenae* COLLECTED IN THE FIELD

Race selfed	Races occurring in F_1 population	Remarks on genotype of race selfed
2	2 (23 cultures)	Homozygous
2	2 (3 cultures)	
3	3 (15 cultures) 4 (8 cultures) 12 (1 culture)	Heterozygous for infection type on Richland
5	2 (11 cultures) 5 (91 cultures) 10 (2 cultures) 12 (2 cultures)	Heterozygous for infection type on Joanette Strain
7	7 (43 cultures)	Homozygous
8	6 (2 cultures) 8 (4 cultures)	Heterozygous for infection type on White Tartar
8	6 (6 cultures) 8 (8 cultures)	Heterozygous for infection type on White Tartar
10*a*	10*a* (25 cultures)	Homozygous

conducted on a small scale, they nevertheless throw some light on the genotypic constitution of the six races selfed. The selfing of two separate cultures of Race 2 suggested that this race is homozygous for pathogenic characters, at least for those that can be distinguished by the reactions of the differential oat varieties used. The selfing of Race 7 and 10*a* also indicated that they were homozygous. Races 3, 5, and 8, however, are clearly heterozygous in respect to their infection types on one or the other of the three differential hosts used.

The pathogenic character of the progeny of the three heterozygous races —Races 3, 5, and 8—is of some interest. The selfing of Race 3 gave rise chiefly to Races 3 and 4. The latter differs from the former only by the infection type on the variety Richland, which is susceptible instead of resistant to this race. In this selfing, therefore, a race to which Richland is resistant has produced a race to which this variety is susceptible. In other words, the small-pustule type appears to be dominant to the large-pustule type. Similarly, in the selfing of Race 8, a race which produces small pustules on White Tartar, there arose a number of cultures of Race 6, a race which produces large pustules on White Tartar, as happened also in the above-mentioned selfing of this race by Gordon and Welsh. This result definitely suggests the dominance, on White Tartar, of the smaller type of pustule.

The selfing of Race 5 would suggest that this race, like Race 2, is homozygous for its infection types on White Tartar and Richland but is heterozygous for the infection type on Joanette Strain. The occurrence of two cultures of

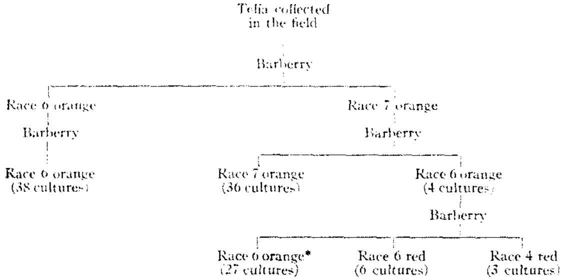
Race 10 and two of Race 12 in the total progeny of 106 cultures may be the result of either a slight admixture of some other race in the selfed culture or a slight amount of accidental intermixture of the pycnial nectar of other races at the time of selfing.

The Selfing of Races of Abnormal Uredial Colour

In addition to selfing studies with races collected in the field, similar studies have been conducted with certain cultures of abnormal uredial colour. As already reported by Gordon (2), two such cultures originated in 1931 from aecia on a barberry plant artificially inoculated with sporidia of teliospores gathered from field plots at Winnipeg in the previous autumn. These cultures, identified as Races 6 and 7, produced uredia described as "ochraceous orange", according to Ridgway's Colour Standards. Selfing studies carried out with these races by the present writers are summarized in the flowsheet.

FLOWSHEET

The selfing of races of abnormal colour



* *3 cultures subepidermal.*

From these studies, it appears probable that Race 6 is homozygous, as 38 cultures from aecia collected at random were all identified as Race 6 orange. Race 7, however, is heterozygous, 36 cultures being identified as Race 7 orange and 4 cultures as Race 6 orange. Subsequently a selfing study of one of the last-mentioned cultures (Race 6 orange) resulted in a progeny composed of 27 cultures of Race 6 orange, 6 cultures of Race 6 red, and 3 cultures of Race 4 red. The unexpected appearance of cultures of normal colour may conceivably be due to mutation, but is more likely the result of an undetected admixture of a race of normal uredial colour. In connection with the selfing, it may be mentioned that three of the orange cultures failed completely to rupture the epidermis of the seedling leaves, a phenomenon never before noted in stem rust of oats although of not infrequent occurrence

in selfed races of stem rust of wheat (4). In relation to the selfing of Race 7 orange, it may be noted that this race is heterozygous whereas, as already pointed out, a field culture of Race 7 gave evidence of being homozygous. The heterozygosity of Race 7 orange resembles that of Race 3 (mentioned above) in that the small-pustule type on Richland is dominant to the large-pustule type. The existence of two genotypically different strains of Race 7 makes it clear that phenotypic similarity, as expressed by the reactions of differential varieties, is not necessarily an index of genotypic identity.

Crosses Between Physiologic Races

Crosses were made between physiologic races of *P. graminis Avenae* with the purpose of comparing the pathogenic characteristics of the F_1 hybrid races with those of the parent races used in the crosses. Such a comparison can be readily obtained by noting the infection types produced by each of the parent races and their hybrid race on the three differential varieties, White Tartar, Richland, and Joanette Strain. If crosses could be made between enough of the races it should be possible to arrive at some generalization as to the inheritance of the pathogenic characteristics expressed through the reaction of these varieties. It should, for example, be possible to determine, in any cross between a race producing small pustules on Richland and a race producing large pustules, whether one of these types is dominant over the other, or whether an intermediate infection type results. If crosses could be made between all of the races differing in this respect and it were found that all hybrid cultures produced a similar infection type, a general rule, applicable to the known races of oat stem rust, might be laid down for the inheritance of this characteristic. The same line of reasoning applies to crosses between races differing in infection type on other differential varieties. As crosses have not yet been made between all of the known races, any generalization made at present must be regarded as more or less tentative.

The methods employed in making crosses need only be described here in brief outline as they have been published previously in detail (6–8). One or more barberry plants are infected sparsely with each of the two races to be crossed. If the pustules resulting from the infection are thinly distributed over the leaves, a considerable proportion will be haploid and hence will fail to develop aecia. When it seems evident that these pustules will not produce aecia if left alone, nectar is transferred from one pustule of one race to several pustules of the other race. As a result of these transfers of pycniospore-bearing nectar, aecia are usually formed in about one-half of the pustules. The aecia thus produced are then used to inoculate oat seedlings of a susceptible variety, after which the physiologic races present in the resulting cultures are identified by means of the differential varieties previously mentioned. The methods described above are illustrated in Fig. 1 by some actual crosses between Races 7 and 10*a*.

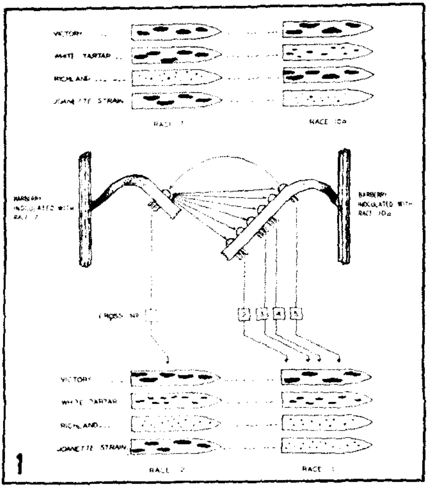


FIG. 1. *A diagrammatic representation of five actual crosses between Race 7 and Race 10a together with the infection types of the parent races and the F_1 hybrid races on the differential varieties. The arrows indicate the direction of transfer of pycnial nectar.*

INHERITANCE OF INFECTION CHARACTERISTICS ON WHITE TARTAR AND RICHLAND

Crosses between Races 2 and 7

These two races differ only by their infection types on the variety White Tartar, on which Race 2 produces small pustules and Race 7 large. The object of crossing them was therefore primarily to discover the infection type of the hybrid race on White Tartar. As each of the parent races was homozygous for the character in question, it was not necessary to make many crosses to decide this point. The results of crosses made in both directions are presented in Table III, A.

As the progeny of the crosses consisted of Races 2 and 5, which produce small pustules on White Tartar, it is evident that the small-pustule type of the Race 2 parent is dominant to the large-pustule type of the Race 7 parent (Fig. 2).

A similar dominance of the small-pustule type on White Tartar was revealed in the previously-mentioned selfing of two different cultures of Race 8, which produces a small type of pustule on this variety. The presence of Race 6,

which produces large pustules in the progeny of both cultures, shows that this type must be recessive to the small-pustule type.

Crosses between Race 5 and Race 7 Orange

Race 5 produces identically the same infection types on White Tartar and Richland as does Race 2, that is, small pustules. Races 5 and 7 differ, therefore, only by their infection types on White Tartar, Race 5 producing the small- and Race 7 the large-pustule type. As in the crosses between Races 2 and 7, the small type of pustule is dominant to the large, so that most of the F_1 progeny (5 out of 7 crosses) produces, on White Tartar, the small pustules characteristic of Race 5. In two of the seven crosses (Table III, B), however, the F_1 progeny was identified as Race 7. As each of these two cultures originated on the Race 7 side of a cross, they might be attributed to accidental

TABLE III

CROSSES BETWEEN RACES OF OAT STEM RUST

---	Races crossed	Number of crosses made	F_1 progeny
A	2* × 7	4	Race 2 (3 crosses) Races 2 and 5 (1 cross)
	7 × 2	3	Race 2 (3 crosses)
B	5 × 7 orange	3	Race 5 (3 crosses)
	7 orange × 5	4	Race 2 (2 crosses) Race 7 (2 crosses)
C	5 × 10*a*	1	Race 5 (1 cross)
	10*a* × 5	2	Race 1 (2 crosses)
D	5 × 6 orange	5	Race 5 (3 crosses) Race 10 (1 cross) Race 12 (1 cross)
	6 orange × 5	9	Race 2 (9 crosses)
E	5 × 8	4	Race 5 (2 crosses) Races 5 and 12 (1 cross) Race 12 (1 cross)
	8 × 5	2	Race 2 (1 cross) Race 10 (1 cross)
F	6 orange × 10*a*	2	Race 8 (2 crosses)
	10*a* × 6 orange	2	Race 10*a* (2 crosses)
G	7 orange × 10*a*	16	Race 2 (7 crosses) Race 8 (9 crosses)
	10*a* × 7 orange	10	Race 1 (6 crosses) Race 10*a* (4 crosses)

* *The race placed first is the race on whose haploid pustules aecia are developed as a result of a transfer to them of pycniospore-containing nectar of the other race.*

selfing of Race 7 orange were it not for the fact that they produce red uredia. a result that suggests that they are in reality the product of crossing. If so, they serve as further proof of the impurity of the culture of Race 5 used in these crosses (see discussion of selfing of Race 5).

Crosses between Race 5 and Race 10a

These two races differ by their infection types on Richland, on which Race 5 produces small and Race 10*a* large pustules. The three crosses made between these two races suggest that the small-pustule type of Race 5 is dominant, as all the hybrid cultures produced this type (Table III, C and Fig. 3).

Crosses between Race 5 and Race 6 Orange

These two races differ by their infection types on both White Tartar and Richland, varieties that are resistant to Race 5 and susceptible to Race 6. As 12 of the 14 crosses resulted in progeny with small pustules (Table III, D and Fig. 4), it appears probable that the small-pustule types of Race 5 are dominant to the large-pustule types of Race 6.

The occurrence of Races 10 and 12 in two of the crosses is interpreted as the result of the impurity of the Race 5 parent.

Crosses between Race 5 and Race 8

Race 8 produces exactly the same infection types on White Tartar and Richland as does Race 10*a*. The crosses between Races 5 and 8 would, therefore, be expected to give results similar to the crosses between Races 5 and 10*a*, in which the small-pustule type of Race 5 on Richland proved dominant to the large-pustule type produced by Race 10*a*. In the crosses between Races 5 and 8, the small-pustule type of Race 5 did, in fact, show dominance to the large-pustule type of Race 8 in five of the six crosses (Table III, E). The occurrence of Race 10 in one cross is perhaps due to the impurity of the Race 5 parent. The unexpected occurrence of Race 12 in two of the crosses may have a similar explanation.

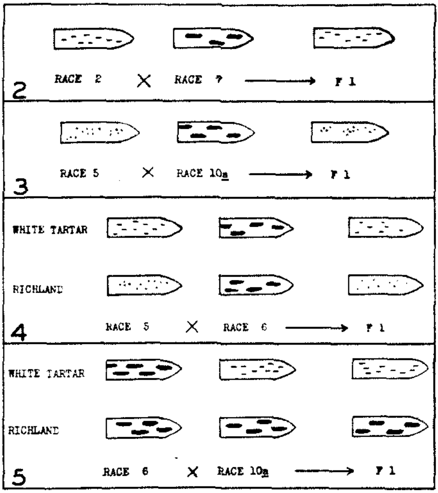
Crosses between Race 6 Orange and Race 10a

These two races differ by their infection types on White Tartar, on which Race 6 produces large and Race 10*a* small pustules. In the four crosses between these two races (Table III, F), the small-pustule type produced by Race 10*a* on White Tartar proved dominant to the large-pustule type produced by Race 6 (Fig. 5).

Crosses between Race 7 Orange and Race 10a

These two races differ by their infection types on White Tartar and Richland. Race 7 produces large pustules on White Tartar and small pustules on Richland, whereas Race 10*a* produces small pustules on White Tartar and large pustules on Richland. The results of crosses between these two races are shown in Table III, G.

The interpretation of the results of the crosses is complicated by the fact that Race 7 orange is heterozygous for its infection type on Richland. A previous selfing of this race (Flowsheet) produced, in addition to Race 7, a number of cultures of Race 6, which develops large pustules on Richland. It would therefore be expected, in any crosses with Race 10*a*, that about one-half of the F_1 cultures would produce large pustules on this variety, whereas, if Race 7 were homozygous, all of the F_1 progeny would produce small pustules. Actually 13 of the 26 F_1 cultures studied produced small pustules and 13 large pustules. The factor governing the small-pustule type of Race 7 on Richland is therefore dominant to that governing the large-pustule type of Race 10*a* on this host.



FIGS. 2–5. FIG. 2. *Infection types on White Tartar of Races 2 and 7 and their F_1 hybrid.* FIG. 3. *Infection types on Richland of Races 5 and 10a and their F_1 hybrid.* FIG. 4. *Infection types on White Tartar and Richland of Races 5 and 6 and their F_1 hybrid.* FIG. 5. *Infection types on White Tartar and Richland of Races 6 and 10a and their F_1 hybrid.*

The results of the crosses, with reference to White Tartar, are simpler to interpret as both parent races were homozygous for their infection types on this variety. All of the 26 F_1 cultures produced the small type of pustule, which proves that, for White Tartar, the small-pustule type of Race 10*a* is dominant to the large-pustule type of Race 7.

Inheritance through the Maternal Cytoplasm

In crosses between certain races of *P. graminis Tritici* Erikss. and Henn., previously described by the writers (7, 9), evidence was obtained that some pathogenic characteristics are inherited through the medium of the cytoplasm of the maternal parent race, that is to say, the race receiving a transfer of pycnial nectar. Where this type of inheritance is manifest, the races arising from the opposite sides of a cross are always different, whereas, if the chromosomes alone functioned, the races should be identical. An examination of Fig. 1 will show that this state of affairs exists in crosses between Races 7 and 10*a*, which produce different infection types on Joanette Strain. The races arising from the Race 7 side of a cross resemble Race 7 in that they produce large pustules on Joanette Strain, while the races arising from the Race 10*a* side of a cross resemble Race 10*a* in that they produce small pustules on this variety. This situation holds true not only for all crosses between Races 7 and 10*a* but apparently also for all crosses in which the two parent races differ in their infection types on Joanette Strain. In almost all such crosses there is a well marked tendency on the part of the hybrid race to resemble the maternal parent race in its infection characteristics on Joanette Strain. In the crosses reported in the present paper, this peculiarity has been noted in crosses between Races 7 and 10*a*, 5 and 7, 5 and 6, 5 and 8, 5 and 10*a*, and 6 and 10*a*. Consequently, in crosses between all these races, the same physiologic race never arises from a cross and its reciprocal cross. Owing to the infection type on Joanette Strain being different, the hybrid races of crosses made reciprocally are always identified as different physiologic races.

This phenomenon does not seem to be capable of explanation on a chromosomal basis, but can be readily explained if it is assumed that the pycniospore nucleus of the paternal race reaches the proto-aecium of the maternal race unaccompanied by its cytoplasm, which, in any case, is very limited on account of the minute size of the pycniospore. Each hybrid aeciospore would then receive from the paternal race only a nucleus, but from the maternal race a nucleus plus cytoplasm. This explanation, according to Lamb (5), "finds an entirely convincing cytological explanation" in his study of the initiation of the dikaryophase in *Puccinia Phragmitis* (Schum.) Körn.

The Inheritance of Urediospore Colour

The fact that, in several of the crosses already described, one race was orange while the other was normal (red) in urediospore colour provided opportunities of studying the inheritance of uredial colour as manifested in the F_1 generation. As the orange races, when selfed, produce uredia of their own colour, it is obvious that any red uredial cultures originating from the orange side of a cross between red and orange races are bound to be hybrids between the two races. The present paper describes crosses between Races 7 orange and 10*a* red, 7 orange and 5 red, 6 orange and 5 red, and 6 orange and 10*a* red. In these crosses, hybrid aecia arising from mycelia of the orange

races have invariably produced red uredial progeny, which proves not only that actual crossing took place but also that red spore colour is dominant to orange. A similar dominance of red to orange spore colour has previously been described for *P. graminis Tritici* (9).

The Effect of Ageing of Teliospores on the Infection Characteristics on the Barberry

In most of the selfing studies reported in this paper, the selfing was carried out within a few months of the formation of the teliospores. The selfing of Race 3, however, forms an exception in that two selfing studies were conducted with the same telial material, the first when the teliospores were about 6 months old, and the second when they were almost 5 years old. In the interval between the two selfings, the teliospores were kept in dry storage at a temperature of about 8 to 10° C. for 4 years and 138 days, namely, from May 15, 1934, to September 30, 1938. During this period of storage, it appears that some change took place in the spores, which resulted in abnormal infection characteristics on the barberry.

In the first selfing, no abnormalities were observed in the infections on the barberry. In the second selfing, however, it was noted that only about half of the infections developed pycnia and pycnial nectar; the remainder had the appearance of small, round necrotic areas frequently surrounded by a purple halo (Plate 1, Fig. 6). When the leaves were cleared with chloral hydrate and stained with acid fuchsin, the mycelia in these necrotic areas showed, in most instances, no indication of even rudimentary pycnial formation (Plate 1, Fig. 7).

A count of the normal and abnormal infections on four different barberry plants showed that nearly half of the infections were of the abnormal type: in a total of 308 infections, 170 were normal and 138 abnormal. These figures suggest that about half of the sporidia were deficient in some respect, so that the mycelia derived from them no longer possessed the ability to develop pycnia. This deficiency appeared to be in no way related to their sex. Both sexes were obviously present among the normal haploid pustules, as aecia formed readily from intermixture of pycnial nectar. That the deficiency was caused by some change in the teliospores during their long period of storage is suggested by the fact that no such abnormalities were noted in the first selfing.

Discussion

The object of the work described in the present paper was, as already stated, to study the inheritance of the pathogenic properties exhibited by physiologic races of *P. graminis Avenae* on the differential varieties used for their identification. While pathogenic properties are in reality physiologic characteristics they have nevertheless a concrete, visible expression, namely, the infection types produced on the leaves of the oat plant. These infection types, the visible manifestations of physiologic potentialities, may, owing to their relative constancy, be considered characters expressing the pathogenicity of the physiologic races.

The two methods of approach to this problem, viz., selfing of individual races and crosses between races, are complementary and yield much the same type of information. The complementary nature of the two methods may be illustrated by considering a definite example such as, for instance, the relation in selfing studies and crosses between the small and the large pustule types on the variety White Tartar. Selfing studies (Table II) have shown that races with small pustules may give rise to races with large pustules, as well as to races with small pustules, whereas races with large pustules have never been known to give rise to races with small pustules. These results therefore suggest the dominance of the small-pustule type. In crosses between Races 2 and 7, and Races 7 and *10a*, the small-pustule type of Races 2 and *10a* is dominant to the large-pustule type of Race 7 in the F_1 progeny. As far as the relationship of these two infection types is concerned, both crosses and selfing studies indicate the dominance of the small type of pustule.

Evidence obtained in a similar way suggests that the small type of pustule on Richland is dominant to the large type. This conclusion is derived from crosses between Races 7 and *10a*, 5 and *10a*, and 5 and 6, and from selfing studies with Race 3 (Table II) and Race 7 orange (Flowsheet), both of which produce small pustules on Richland but, when selfed, give rise to races with large as well as small pustules.

If, as seems quite possible, these facts are applicable to oat stem rust in general, they may help to throw some light on the role played by the barberry in the development of physiologic races. Owing to the dominance of the small-pustule types, it is always possible for the less virulent physiologic races, if they occur in a heterozygous state, to produce, through natural selfing, races of greater virulence on the newly developed rust-resistant varieties, most of which react to stem rust like either Richland or White Tartar. But, for exactly the same reason, natural crosses of the less virulent with the more virulent races are likely to produce hybrid races resembling the less virulent of the two races entering into a cross. It would seem, therefore, that there are two opposing processes at work, one of which, selfing, tends to bring into being strains of rust with greater virulence than the selfed parent race, while the other, crossing, tends to submerge the more virulent, recessive characteristics of the rust. Perhaps, in the long run, the two opposing processes will balance each other so that there may not be any significant increase in the virulence of oat stem rust through the medium of the barberry.

Nothing much can be said, at present, concerning the mechanism of the inheritance of the pathogenic properties discussed above. The presence of the phenomena of dominance and recessiveness suggests a chromosomal basis and a Mendelian inheritance, but in view of the small populations investigated in the selfing studies it is not possible to place this inheritance on a factorial basis. That the inheritance of pathogenic characteristics is not entirely chromosomal is shown by the apparent influence of the cytoplasm of the maternal race on the infection type on Joanette Strain. As a result, the infection type of the hybrid race on Joanette Strain closely resembles that of the

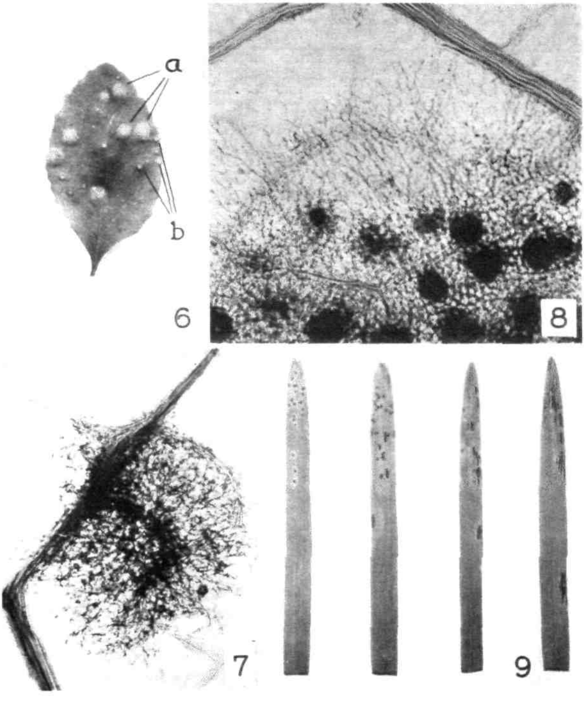


PLATE I. FIGS. 6–9. FIG. 6. *A leaf of Berberis vulgaris showing (a) normal infections (b) and abnormal infections produced by Race 3 after the teliospores had been kept in storage for more than 4 years.* FIG. 7. *Mycelium of an abnormal haploid pustule 24 days after inoculation.* ×650. FIG. 8. *Mycelium of a normal haploid pustule 24 days after infection, showing proto-aecia.* ×650. FIG. 9. *Infection types produced by P. graminis Avenae on seedling leaves of the differential oat varieties used for the identification of physiologic races. From left to ri ht: types 1 2, x, 4.*

maternal parent race. Exactly the same phenomenon has been noted previously in crosses between physiologic races of *P. graminis Tritici* (7, 9), the influence of the cytoplasm there being discernible only in the infection types on the varieties Marquis and Kota.

In general, there is a rather close parallelism between the inheritance of pathogenic and other characters in *P. graminis Avenae* and *P. graminis Tritici*. In both rusts certain pathogenic properties are dominant to others; in both normal (red) spore colour is dominant to the atypical orange colour; and in both there is evidence of the influence of the cytoplasm on inheritance. While the effect of inbreeding on races of oat stem rust has not been studied extensively, there is some evidence that it leads to the development of strains with abnormal characteristics such as atypical colour and lack of vigour of sporulation. The appearance, after the third consecutive selfing (Flowsheet), of strains with uredia that failed to rupture the epidermis of the oat seedling leaf would suggest that, as in wheat stem rust, repeated selfings tend to lower the survival value of the rust.

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CALYX END STRUCTURE IN THE GRAVENSTEIN APPLE[1]

By Hugh P. Bell[2]

Abstract

The development and structure of the calyx end of the Gravenstein is compared with that of a number of other varieties. This comparison indicates:—(i) that the calyx end of the Gravenstein exhibits an unusual degree of variation in its mode of development; (ii) that at maturity it is usually composed of a tissue which is exceptionally fissured and porous; (iii) that most of these fissures and pores are radial slits; and (iv) that, as these openings are so unusually large and numerous in the Gravenstein, its calyx end must be structurally weaker than the corresponding structure in the other varieties. It is suggested that the prevalence of open core in the Gravenstein is due to this structural weakness of the calyx end.

Introduction

The studies of mouldy core of the Gravenstein apple carried out by Brittain and Eidt (1) and by Harrison (2) established a definite relationship between mouldy core and open core. Harrison (2, p. 367) put forward the suggestion that the prevalence of open core in the Gravenstein was due to the "constitutional weakness of the calyx end" in this variety. The present work was undertaken to determine whether the calyx end of the Gravenstein was structurally weaker than that of other varieties of commercially grown apples.

Collections and Technique

The development of the calyx end of the Banks Gravenstein was traced from November 1937 until September 1938. The varieties selected for comparison were Ben Davis, Stark, and McIntosh Red. Collections from these and the Banks Gravenstein were made once a month from November to February, twice during March and once a week from April to the end of August. Also, from blossom time during the first week of June until the end of August, weekly collections were made from the Blenheim, Crimson Gravenstein, Macoun, King, Delicious, Winesap, Bramley Seedling, Evangeline, and Ortley (Cleopatra). Permanent slides were made from all these collections and included both transverse and longitudinal serial sections through the calyx end of ovaries or young fruit. The usual paraffin methods were employed. The serial collections for each variety were taken from one tree only. For each collection a tree was selected that appeared to be normal in every way.

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Contribution from the Department of Biology, Dalhousie University, Halifax, Nova Scotia, with financial assistance from the National Research Council of Canada.
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To be sure that each tree was normal and typical of its variety, young fruit was collected from various other trees of the same variety. This material and that from the selected trees were compared while fresh by means of hand sections. Besides checking the suitability of the selected trees, the hand sections of fresh material provided information which could not be obtained from the permanent slides. In addition, valuable material was kindly loaned by Mr. K. A. Harrison of the staff of the Laboratory of Plant Pathology, Kentville, N.S. This consisted of collections from the varieties Ortley, Banks Gravenstein, Gordon Gravenstein, Stark, Evangeline, and Wagener. In 1938 the period of full bloom for the orchard at the Kentville Experimental Station was about June 2.

Nomenclature

In Harrison's paper (2, p. 359), the terms describing the structural units at the calyx end are clearly defined. Three of his definitions are as follows:—

Calyx tube, sometimes called "Calyx cup." The cavity formed by the union of the bases of the sepals and extending from the sepals to the point of union of pistil and flesh.

Style tube. A passageway through the centre of the style that connects the core with the calyx tube. This was described by Miss Tetley from the variety Bramley's Seedling.

Open core. A core with an opening connecting it with the "calyx tube". Includes all breaks in the continuity of the tissue that permit penetration by saprophytic fungi. Replaces "open calyx tube" because it is considered more descriptive and cannot be confused with "open calyx".

These definitions have been followed in the present paper. Also the terms "up," "upward", and "above" in descriptions of flower or fruit structure have reference to distal portions and similarly "down", "downward", and "below" refer to proximal portions.

Structures and Period of Development to be Described

The normal apple at all stages of growth is excellently described by Kraus (3, 4), so it is unnecessary to repeat his descriptions. After examination of the early collections, it was apparent that there was no need to discuss the development that occurs before the second week of May or to deal with any structures other than those at the calyx end. To be more explicit, this article deals only with the part of the ovary between the carpellary cavities and calyx tube and traces the development of this portion from just before the middle of May until the fruit is mature in the autumn.

Comparison of Development

Early Development for All Varieties

About the second week of May, that is, three weeks before the time o full bloom, the structure of the calyx end of the young flower is fundamentall similar for all varieties of apple studied. At that time, the top of the ovar

consists of a cylinder of tissue, through the centre of which runs the open style tube. This style tube is typical and practically the same for all varieties (Fig. 2). It is usually oval in cross section but sometimes round and from it radiate ten vertical slits. Five of these are upward continuations of the carpellary cavities (*d*, Fig. 1), and may be recognized by the presence of the dorsal carpellary bundle. The five alternating with these are downward continuations of the spaces between the styles (*c*, Fig. 1). During the early development, the upper portions of the former and the lower portions of the latter become closed by the subtending surfaces of epidermal cells coming together, but the original locations of the radial fissures are indicated by ten double rows of epidermal cells radiating from the style tube. Sometimes the two sets of radial openings overlap, but usually, by the middle of May, only one set occurs as openings at any one level. Fig. 2 is of a section through the upper portion of the style tube. In this the continuations of the spaces between the styles occur as five radial fissures, but the continuations of the subtending lining surfaces in the carpellary cavities are in contact. The style tube at this time is continuous, and has an average transverse measurement at its narrowest part of 150 by 40 μ.

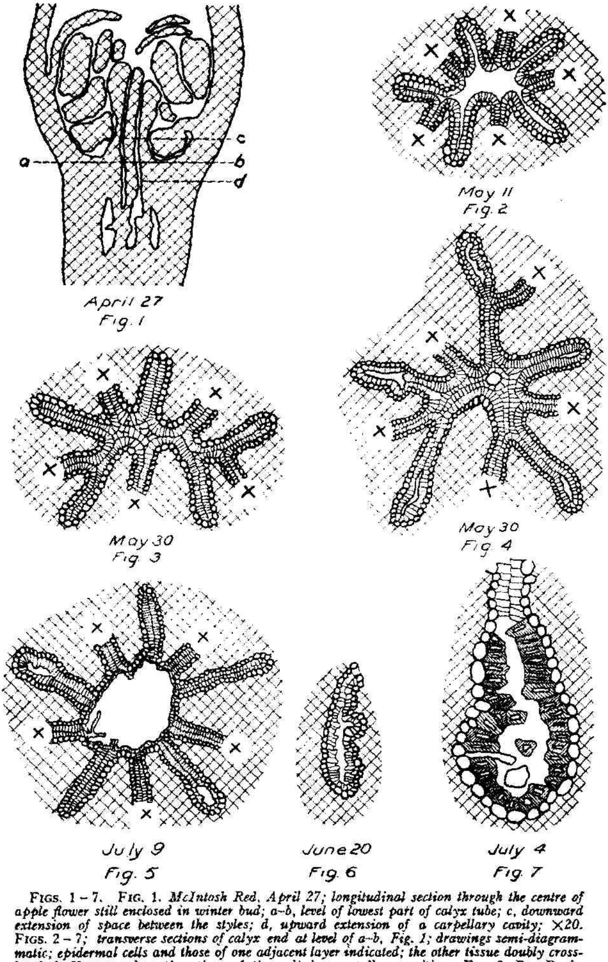
During the three weeks preceding full bloom, growth at the calyx end of the apple is such that there is a tendency for part of the passage provided by the style tube to become closed. The place where the closing begins is about on a level with the lowest part of the calyx tube or calyx cup (*a-b*, Fig. 1). This consists of a depressed circular groove around the united style bases. It is on a level with the bottom of this groove that the closing of the style tube first occurs.

Later Development in Varieties Used as a Check

Shortly before and during blossom time, the development varies from variety to variety; but, with the exception of the Gravenstein, it is quite constant for each variety. The different modes of development produce different types of calyx end structures. These may be grouped into three classes, namely:— (I) those with a completely closed style tube; (II) those with a central opening or style tube persisting; and (III) those with the style tube closed but the surfaces of some of the radial slits failing to fuse, leaving a varying number of radial openings.

The first type, with complete union of all tissues and hence complete closing of the style tube, is well represented by the Stark and Ben Davis and is illustrated in Fig. 3. Throughout June and even into July the united epidermal layers may still be recognized, but subsequent growth of cells makes their recognition difficult. After the style tube was apparently closed, very thin hand sections of fresh material were cut and an attempt made to separate the tissues along the original line of union, but no separation could be affected. From this it may be concluded that in these varieties the union of cells is complete.

The second type, with an open style tube in the centre, is well represented by Bramley Seedling, and is described by Tetley (5). Other varieties in this



FIGS. 1 – 7. FIG. 1. *McIntosh Red, April 27; longitudinal section through the centre of apple flower still enclosed in winter bud; a–b, level of lowest part of calyx tube; c, downward extension of space between the styles; d, upward extension of a carpellary cavity;* ×*20.* FIGS. 2 – 7; *transverse sections of calyx end at level of a–b, Fig. 1; drawings semi-diagrammatic; epidermal cells and those of one adjacent layer indicated; the other tissue doubly cross-hatched; X, upward continuations of tissue lining carpellary cavities.* FIG. 2. *Ben Davis, May 11; style tube still open;* ×*105.* FIG. 3. *Stark, May 30; style tube closed;* ×*105.* FIG. 4. *Banks Gravenstein, May 30; downward continuations of spaces between styles open, forming radial fissures, also a small open style tube;* ×*105.* FIG. 5. *Crimson Gravenstein, July 9; a large open style tube, circular in cross section, also two small radial openings;* ×*105.* FIG. 6. *Banks Gravenstein, June 20; an open radial fissure similar to those in Fig. 4, but more advanced; epidermal cells elongating and walls of some thickening;* ×*105.* FIG. 7. *Banks Gravenstein, July 4; as in Fig. 6, but a later stage with walls of all epidermal cells lignified;* ×*385.*

class are Evangeline and Delicious. The style tube in these does not always remain circular in cross section. It is more frequently oval and at times it may divide into two or three tubes separated by thin partitions. Fig. 5 illustrates a calyx end with an open style tube that is circular in cross section. The open style tube is sometimes accompanied by small radial fissures (Fig. 5), but the central opening is larger and more prominent, and it differs from the others in that it provides a continuous open passage from the outside to the inside of the fruit.

The varieties of the third class, that is, those with the style tube closed but the surfaces of some of the radial slits failing to fuse, are fairly numerous and include the McIntosh Red and the Wagener. The failure to close is similar to, but usually less extensive than that illustrated for the Gravenstein in Fig. 4. Lack of fusion is much more common in the downward continuations of the spaces between the styles than in the upward continuations of the carpellary cavities. The fissures observed by Harrison in the Wagener (2, p. 366) were without doubt these partially closed radial slits. These radial apertures are at first lined by delicate epidermal cells, but as the fruit matures, these lining cells either grow into short stiff hairs or, by elongating uniformly at right angles to the surface and depositing a secondary thickening on the wall, form a layer of semi-columnar but distorted thick-walled cells. The hairs are more common for the central opening and the layer of thick-walled cells for the peripheral part of the radial opening, but they may be interspersed. In either case the cells become mature and inactive, so that tissue union between these subtending surfaces does not take place, for in the mature fruit the radial apertures are still present. These openings alone do not provide a passageway between the outside and the inside of the fruit, but, as partially closed and laterally located pores or pockets, are present in the tissue at the calyx end in a large number of varieties.

Later Development in the Gravenstein

The Gravenstein is different from the other varieties in two ways. First, there is a lack of uniformity in the method of growth, and second, when radial openings occur, they are usually larger and more numerous.

Sometimes the Gravenstein apple closes perfectly as in Class I (Fig. 3). Occasionally a continuous central style tube persists as in Class II (Fig. 5). This latter condition, however, is comparatively rare.

The majority of Gravensteins belong to Class III, having openings of varying sizes in some of the ten radial slits (Fig. 4). As in the other varieties, these spaces occur much more commonly in the downward extensions of the spaces between the styles than in the upward extensions of the carpellary cavities, but they are by no means uncommon in the latter. There is no uniformity in the number of slits that remain unclosed but it is seldom less than three, and occasionally some opening may be found in all ten rays. Sometimes the style tube closes completely, although large laterally placed radial apertures may be present in the same fruit. Sometimes the radial fissures are accompanied

by a small or large style tube; but usually a small style tube such as that illustrated in Fig. 4 is closed for a short distance, although it may remain as a very narrow passage through most of the tissue at the calyx end. The radial openings vary greatly in size. Their horizontal length varies from practically the whole extent of the ray (Fig. 4) to that of a small oval aperture at the distal end (Fig. 5). Also in vertical extent, they vary from a small closed pocket to a continuous vertical passage closed at one end only. But consideration of these great variations must not obscure the constant feature, namely, that in the Gravenstein these radial openings are unusually extensive and numerous.

It cannot be assumed, however, that because these radial openings are so large and numerous, that they provide a passageway from the outside into the carpellary cavity. In this respect the Gravenstein is not different from the other varieties. The downward continuations of the spaces between the styles usually close at the level of the top of the carpellary cavities proper and the upward continuations of the carpellary cavities usually close below the level of the base of the styles. In the intervening region, that is, between the top of the carpellary cavities and the base of the calyx tube, these two sets of openings may overlap and give to the tissue there a fissured or porous character, which must be a great source of weakness; yet, unless these radial openings connect with a central style tube, they, by themselves, do not provide a continuous passage from the outside to the inside.

As already stated, the radial fissures or pores do not close during the maturing of the fruit, but the lining cells grow into either hairs or elongated thick-walled cells. These thick-walled cells were conspicuous in the Gravenstein, and their progressive development is illustrated in Figs. 6 and 7. But it is evident that this thickening of the walls of the individual cells does not add to the general rigidity of the region, for the structural weakness of the tissue is the long open slits, and these are to be found even in the ripened fruit. Also, as the fruit enlarges, the soft surrounding cells tend to tear away from this stiff or rigid lining, producing additional openings. Thus instead of the thick-walled cells giving any strength to the tissue, they may be a source of weakness.

Using mature Gravenstein apples in which a split at the calyx end had produced an open core, an attempt was made to locate the origin and exact position of the split. It was found that these breaks did not always pass through the exact centre where the original style tube would be located, but their location might be slightly excentric or lateral through the tissue which would be occupied by the radial apertures. When once the fruit had cracked open, the tissues were so torn and otherwise changed that one could not be certain that the break had followed the line of an open radial fissure, but all the evidence suggested that this was the case.

Final Comparison

A comparison of the Gravenstein with the other varieties examined, indicates:— (i) that the calyx end of the Gravenstein exhibits an unusual degree of variation in its mode of development; (ii) that at maturity it is usually composed of a tissue which is exceptionally fissured and porous; (iii) that most of these fissures and pores are radial slits; and (iv) that, as these openings are so unusually large and numerous in the Gravenstein, its calyx end must be structurally weaker than the corresponding structure in the other varieties.

Discussion

The morphological evidence presented above parallels and partially explains the evidence of a different type presented by previous investigators. The large percentage of open core for the Gravenstein reported by both Brittain and Eidt (1) and Harrison (2) would be expected in a variety with such an exceptionally weak calyx end. The more frequent appearance of open core in the larger apple (2, p. 364) may be accounted for, since the larger the size attained, the greater the growth strains and the greater the likelihood of a break where weakness exists. That is, the fact that so many Gravensteins have a calyx end which is structurally weaker than that of other varieties explains why open core is so common in the Gravenstein, but still leaves unexplained why radial fissures close in some apples and remain open in others from the same tree.

Conclusion

The logical conclusion from the findings outlined above is that Harrison (2, p. 367) is correct when he suggests that open core in the Gravenstein is correlated with a "constitutional weakness of the calyx end" of that variety.

Acknowledgments

The author wishes to express his indebtedness to the Pathologist-in-Charge, Laboratory of Plant Pathology, Kentville, Nova Scotia, for laboratory space during two summers, and to the staff of the laboratory at Kentville for collections made throughout the winter of 1937–38. The figures were drawn by Miss Elizabeth E. Bligh, of Kentville, N.S.

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PHYSIOLOGIC RACES OF *USTILAGO HORDEI* (PERS.) K. AND S. IN ALBERTA[1]

BY WM. SEMENIUK[2]

Abstract

The pathogenicity of four collections of *U. hordei* on four varieties of barley was determined in 1935, 1937, and 1938. Investigations conducted in 1935, involving 12 collections, indicated that the four used in the present investigation represented distinct physiologic races. The distinctions between these races appeared to be substantiated in 1937, but the 1938 results were conflicting. One race (*B*) is clearly distinct from the other three (*F*, *H*, and *L*), but the cause of the erratic behaviour of *F*, *H*, and *L* from year to year is obscure.

Introduction

Resistance to covered smut (*Ustilago hordei* (Pers.) K. and S.) and loose smut (*U. nuda* (Jens.) K. and S.) is being emphasized in the barley breeding work in progress at the University of Alberta. This paper is concerned only with the results of investigations on physiologic specialization in *U. hordei*. Results of investigations on comparative varietal reactions will be published later.

The existence of physiologic races in *U. hordei* has been reported by several investigators (1–5). Owing largely to difficulties in securing heavy infections and to a lack of agreement between results obtained in different years, some of the data published are little more than suggestive. Probably the most convincing evidence is to be found in a paper by Tapke (5), in which eight physiologic races are clearly distinguished. Striking differences in cultural characteristics have been demonstrated by Rodenhiser (4), and two of the cultural strains were found to differ in pathogenicity.

Material and Methods

In 1934, 12 collections of *U. hordei* were obtained from widely separated points in Alberta. Each collection consisted of a bulk sample of chlamydospores obtained from several infected spikes in one field. Thirteen varieties were inoculated with each of the 12 collections and grown in 1935. The results of this preliminary trial indicated that four of the collections differed with respect to their ability to infect the varieties Colsess, O.A.C. 21, Hannchen, and Trebi. These collections and the four varieties named provided material for further experiments.

To obtain inoculum for succeeding experiments, infected spikes, representing each collection, were gathered from each of the four varieties except, of course, from varieties on which no smut developed. The identity of the collections

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was carefully maintained, but samples of individual collections secured from different varieties were bulked. In other words, no attempt was made to effect a separation of biotypes within a collection by means of the possible "screening" action of the different varieties.

The chlamydospores were threshed free by rubbing the infected spikes on wire cloth with a glass rod. The cloth and rod were sterilized before threshing each collection.

In all tests, the seed was partially dehulled before inoculation. The embryo was exposed by removing the lower part of the lemma with a small knife after soaking the seeds in tap water for about 20 min.

In 1935, the material was planted in eight-foot rows at the rate of 75 seeds per row. In 1937, only 50 seeds were planted in each row. In 1938, the rows were 10 ft. in length with 100 seeds in each row. Varieties and collections were randomized within each of the two replicates. When classifying the material, counts were made on a plant basis and no distinction was made between partially and completely infected plants.

The test was planted in 1936 also, but the degree of infection was so low that the results were worthless.

Experimental Results

The percentage infections by the four collections on four varieties and the numbers of plants on which the percentages are based are recorded in Table I. Each entry in the table represents a total of two replicates.

TABLE I

TOTAL PLANTS OF FOUR VARIETIES OF BARLEY AND PERCENTAGE INFECTION BY FOUR COLLECTIONS OF *Ustilago hordei*

Collection	Source	Year tested	Total plants (two replicates)				Percentage infected			
			Colsess	O.A.C. 21	Hannchen	Trebi	Colsess	O.A.C 21	Hannchen	Trebi
B	Innisfree, Alberta	1935	104	86	106	112	2.9	0.0	52.8	52.7
		1937	47	54	31	50	4.2	0.0	25.8	46.0
		1938	159	161	122	192	3.8	0.0	67.2	49.5
F	Cooking Lake, Alberta	1935	93	24	119	71	30.1	8.3	0.8	0.0
		1937	46	51	28	38	17.4	0.0	0.0	0.0
		1938	156	155	113	171	63.5	1.9	65.5	59.1
H	Alaska	1935	123	94	117	121	13.8	13.8	0.0	0.8
		1937	59	57	41	62	0.0	8.8	0.0	0.0
		1938	163	163	167	179	43.6	3.1	40.1	58.6
L	Edmonton, Alberta	1935	133	141	132	130	36.1	5.7	48.5	35.4
		1937	47	51	20	42	51.1	0.0	30.0	23.8
		1938	168	164	164	165	63.7	0.0	41.5	60.6

On the basis of the data assembled in 1935 and 1937, the four collections may be distinguished by means of their relative ability to infect Colsess, O.A.C. 21, and either Hannchen or Trebi. The 1937 results agree very well with those obtained in 1935 except in the cases of collection *F* on O.A.C. 21 and collection *H* on Colsess. In 1938, however, the distinctions between collections *F*, *H*, and *L* practically disappeared. The identity of collection *B* was maintained by its inability to produce more than a slight infection on Colsess.

Discussion

Perhaps the most interesting, though disconcerting, feature of the results presented is the extent to which the classification of smut collections, based on 1935 and 1937 results, is altered by the 1938 results. The cause of the sudden change in pathogenicity in 1938 is obscure. It is probable that most, if not all, races of *U. hordei* are highly heterozygous with respect to pathogenic capabilities. Since segregation and recombination are involved in each generation of chlamydospores, complete uniformity of results from year to year is not to be expected. However, the segregation hypothesis is hardly likely to explain the difference between the 1935 and 1937 data and those obtained in 1938. The remarkable uniformity from year to year, of the data compiled by Tapke (5) is of interest in this connection. It is possible that seasonal differences in the environment may have favoured the multiplication of certain biotypes within the collections. This suggestion is, however, pure speculation.

Acknowledgments

Mr. W. H. Johnston, now Assistant Superintendent of the Dominion Experimental Farm, Brandon, Manitoba, secured the collections of smut and directed the 1935 tests. His contribution is gratefully acknowledged. The author is also indebted to Dr. K. W. Neatby, Professor of Field Crops, University of Alberta, for his assistance in the preparation of the manuscript.

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RELATION OF SUNSPOT PERIODICITY TO PRECIPITATION, TEMPERATURE, AND CROP YIELDS IN ALBERTA AND SASKATCHEWAN[1]

By L. P. V. Johnson[2]

Abstract

Coefficients of correlation (*r*) were calculated for sunspot data as related to annual precipitation and to temperature data from Edmonton, Calgary, Medicine Hat, Swift Current, Battleford, and Qu'Appelle; and as related to data on the yields of wheat, oats, and barley in Alberta and Saskatchewan. In all cases the correlation was negative, but only in the cases of precipitation at Edmonton, Calgary, and Battleford were the values significant. Data giving significant values of *r* are given graphical treatment.

It was concluded that while the sunspot cycle probably bears some causal relation to precipitation, and perhaps also to temperature and indirectly to crop yields, it, nevertheless, does not provide a basis for long-range weather forecasting owing to the confusing effects of other weather-determining factors.

The literature on sunspot periodicity in relation to terrestrial phenomena is reviewed briefly.

Introduction

The extent to which sunspot periodicity influences weather has been a controversial point for many years. Recently this controversy has become more active, owing, no doubt, to the increased popular interest in seasonal weather predictions, which has been occasioned by the recent widespread droughts on the Great Plains of North America.

It is the writer's opinion that many, if not most, of the differences in this dispute have arisen through the methods of data analysis used by some authors in attempting to establish high correlations between the variables. For example, the use of smoothed mean graphs (representing the average of the superimposed graphs of data from several cycles or periods of rise and fall) greatly exaggerates the true relation between the variables with respect to individual events of coincidental occurrence. Such exaggeration has led to over-emphasis, which, in turn, has added fuel to the controversial fire.

The present study represents the application of the coefficient of correlation and direct graphical methods to unmanipulated data in investigating the effective, directly apparent influence of sunspot periodicity on precipitation, temperature, and crop yields in Alberta and Saskatchewan.

Periodicity in Number of Sunspots

The number of observed sunspots varies greatly from year to year, showing a primary periodicity of about 11 years (Fig. 1 and Tables I, II, and III). Besides this primary periodicity a number of other cycles have been established,

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notably that known as the double, Hale, or 22-to-23-year cycle. This cycle represents essentially an alternate variation in the intensity of sunspot maxima. Reference to Fig. 1 will serve to demonstrate that the alternate maxima of 1870, 1893, 1917, and 1937 are each higher than the maximum preceding. In this paper we shall designate these maxima as major and minor, respectively.

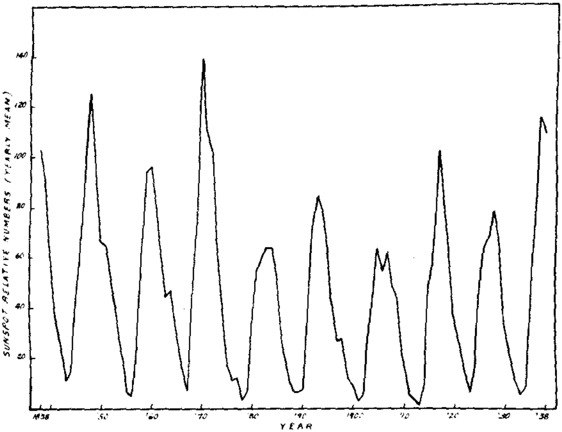

FIG. 1. *Cyclic variations in yearly mean sunspot relative numbers for the period 1838–1938.*

Attempts have been made to group major and minor maxima in various other ways. But since we have reasonably accurate data on variability of sunspot numbers for less than 200 years, the groupings of longer intervals are difficult to establish. However, it would appear safe to assume from the standard Wolf-Wolfer-Brunner data on sunspot relative numbers for the interval 1749–1938 that there are definite quiet and active periods. Quiet periods existed during the intervals, ?–1766, 1798–1833, 1878–1933; while active periods held during the intervals, 1766–1798, 1833–1878 with anothe commencing (possibly) in 1933.

Fig. 1 shows two major maxima and one minor maximum of the activ period ending in 1878, and three major and three minor maxima of the quie period 1878–1933. It will be noted that since the first maximum of the quie period (1883) there has been a general upward trend in both major and mino maxima.

In Tables I, II, and III, references to major maxima are printed in capital

Effect of Sunspot Periodicity upon Terrestrial Phenomena

It is an established fact that periodic variations in numbers of sunspots produce appreciable effects upon certain electro-magnetic phenomena on the earth, e.g., the number and magnitude of magnetic disturbances, the brilliancy of aurorae, the strength of electric currents in the earth's crust, etc. (2, 16, 18).

The influence of sunspot periodicity on terrestrial weather, however, is much less apparent and, because of much conflicting data (possibly misinterpreted), the relation can hardly be said to be generally accepted as an established fact. In 1929 Dr. Carpenter (3) made the statement that "the verdict of the Mount Wilson Solar Observatory astronomers is that they can find *no relationship between sunspots and weather*" (italics mine). Some authors on this subject (2, 15, 16, 18) conclude that, while sunspots may have some effect, the influence is worthless as a basis of weather forecasting. Others (1, 5–14) take the stand that the sunspot cycle determines weather to an extent sufficient to warrant certain types of prediction. Certain members of the latter group claim that sunspots, operating through their effect on weather, have unmistakable influences on crop yields (9), tree growth (1, 9, 11, 12), fish catches (9), abundance and health of insects, birds, and wild quadrupeds (7, 9, 10), levels and times of freezing and break-up of ice in lakes and rivers (9), commodity prices (9), marriage rates (9), etc.

The means by which sunspots influence terrestrial phenomena is not definitely known. DeLury and co-workers (9, 10) state that emission of ultra-violet light from the sun varies directly with the number of sunspots. Since ultra-violet light is thought to ionize the upper terrestrial atmosphere, it is considered that liberated ions diffusing downward would cause electro-magnetic and meteorological changes observable on the earth. Thomson (18) declares, however, that there is no correlation between numbers of sunspots and intensity of ultra-violet radiation.

It should be stated that DeLury (8, 9) has found that in oceanic regions precipitation is greater at sunspot maximum (aquene response), while in inland regions precipitation is less at sunspot maximum (terrene response). Certain regions near the coast exhibit an intermediate or terraquene response in which aquene and terrene responses are blended in various ways. These responses have not been satisfactorily explained. Obviously, in making correlative studies on sunspots and precipitation, it is necessary to deal separately with data from regions of opposing response.

Correlation of Sunspot Periodicity with Precipitation, Temperature, and Crop Yield in Alberta and Saskatchewan

The great extent to which agriculture in the semi-arid region of Alberta and Saskatchewan is dependent upon the weather was emphasized by recent drought conditions. This fact prompted the present attempt to investigate possible influences of sunspot periodicity upon precipitation and temperature in the region. It has been possible to extend the study to include also the

influence upon crop production, since precipitation to a large extent and temperature to a lesser extent determine yields in the area.

Data were obtained on total annual precipitation and mean annual temperature at six meteorological stations situated in the semi-arid region. These stations, with date of first available data, are as follows: Edmonton, 1883; Calgary, 1885; Medicine Hat, 1884; Swift Current, 1886; Battleford, 1891; Qu'Appelle, 1884. The period from the dates given to 1938, inclusive, covers approximately five 11-year sunspot cycles.

Data were obtained on annual yield per acre of wheat, oats, and barley for the years 1898 to 1938, inclusive, a period covering somewhat less than four 11-year sunspot cycles.

(a) Source of data

Sunspot periodicity: Data supplied by Dr. R. E. DeLury, Ottawa. Solar Observations, Dominion Observatory, Ottawa.

Precipitation: Data sheets supplied by Mr. J. Patterson, Controller, Meteorological Division, Air Services, Department of Transport, Toronto.

Report of Conference on Conservation of Soil Fertility and Soil Fibre. Winnipeg, July 14–16, 1920.

Temperature: Data sheets supplied by Mr. J. Patterson, Toronto.

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Annual Report, Secretary of Statistics, Dept. Agr., Sask., 1913, 1914, 1915, 1929 (summarizes data 1905–1928), and 1938 (summarizes data 1923–1937).

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General. Unpublished tables and work sheets supplied by Chas. F. Wilson, Statistician, Agricultural Branch, Dominion Bureau of Statistics, Ottawa.

(b) Precipitation–sunspot correlations

In Table I, yearly mean relative sunspot numbers are compared with total annual precipitation at the meteorological stations situated at Edmonton, Calgary, Medicine Hat, Swift Current, Battleford, and Qu'Appelle. The

TABLE I

YEARLY MEAN SUNSPOT RELATIVE NUMBERS COMPARED WITH PRECIPITATION DATA FROM SIX POINTS IN ALBERTA AND SASKATCHEWAN

Year	Sunspot relative number	Total annual precipitation in inches at						Mean of all points
		Edmonton	Calgary	Medicine Hat	Swift Current	Battleford	Qu Appelle	
1883	63.7 max.	9.27	—	—	—	—	—	—
84	63.5	15.67	—	14.53	—	—	13.97	—
85	52.2	15.36	13.67	8.65	—	—	11.92	—
86	25.4	9.22	11.32	6.72	10.77	—	10.14	—
87	13.1	12.50	13.69	9.89	18.00	—	14.47	—
88	6.8	19.93	17.51	14.67	14.09	—	17.00	—
89	6.3 min.	8.16	11.59	7.96	10.46	—	10.54	—
1890	7.1	22.01	15.47	9.13	17.50	—	23.97	—
91	35.6	17.90	10.44	13.15	24.55	9.35	19.02	15.73
92	73.0	16.85	7.91	12.24	20.20	11.06	16.45	14.12
93	84.9 MAX.	17.87	11.05	14.60	14.54	10.93	16.35	14.52
94	78.0	16.13	11.70	13.14	9.66	13.47	12.52	12.77
95	64.0	14.68	15.12	14.13	12.33	12.01	15.29	13.93
96	41.8	15.24	16.05	18.18	14.11	12.93	21.63	16.36
97	26.2	14.55	20.58	17.27	16.24	16.53	12.65	16.30
98	26.7	10.90	16.21	15.90	15.25	14.25	21.65	15.69
99	12.1	20.89	26.15	22.28	19.38	18.42	19.27	21.06
1900	9.5	27.81	17.57	22.05	14.60	20.37	16.52	19.82
01	2.7 min.	27.51	22.31	20.80	18.58	16.07	26.47	21.96
02	5.0	20.86	34.57	13.68	17.64	13.49	24.37	20.77
03	24.4	21.06	22.77	9.90	18.38	16.06	20.09	18.04
04	42.0	18.87	11.89	9.70	12.84	16.60	22.22	15.35
05	63.5 max.	15.56	14.32	8.99	15.68	10.55	24.55	14.94
06	53.8	19.00	16.24	12.62	19.02	10.64	20.39	16.32
07	62.0	9.88	14.96	6.86	13.17	10.11	18.53	12.25
08	48.5	17.89	18.25	10.22	12.60	17.51	18.67	15.86
09	43.9	12.88	16.03	9.80	19.26	12.02	25.75	15.96
1910	18.6	14.93	12.03	7.55	10.13	8.75	19.02	12.07
11	5.7	20.67	19.47	16.14	14.29	20.47	20.61	18.61
12	3.8	20.18	21.32	10.38	14.62	14.84	18.06	16.57
13	1.4 min.	19.54	17.03	13.62	12.60	11.73	21.24	15.96
14	9.6	25.29	17.70	12.17	12.48	19.14	19.77	17.76
15	47.4	18.64	18.32	16.13	14.27	8.69	18.67	15.79
16	57.1	20.92	13.91	17.90	23.98	17.73	26.54	20.16
17	103.9 MAX.	15.25	11.44	11.13	11.92	8.20	16.69	12.44
18	80.6	17.86	9.12	10.01	12.22	9.76	15.53	12.42
19	63.6	16.43	11.98	7.64	12.33	10.26	17.92	12.76
1920	37.6	18.16	14.42	10.74	10.23	19.64	19.72	15.48
21	26.1	15.22	13.48	12.94	15.05	19.48	27.19	17.23
22	14.2	13.77	10.61	11.54	14.27	10.95	22.03	13.86
23	5.8 min.	17.44	23.87	15.46	16.38	14.70	27.05	19.15
24	16.7	18.77	24.29	9.88	16.38	10.38	16.45	16.02
25	44.3	17.44	18.06	16.17	14.33	14.59	16.70	16.21
26	63.9	15.05	24.36	11.90	15.88	11.30	19.30	16.30
27	69.0	17.68	29.84	25.28	23.13	13.63	25.55	22.52
28	77.8 max.	15.15	16.64	7.64	11.77	10.66	12.07	12.32
29	65.0	15.12	14.47	9.32	14.86	9.82	12.05	12.61
1930	35.7	12.40	14.49	12.73	13.54	14.64	13.16	13.49
31	21.2	20.04	11.84	9.96	11.87	7.28	10.72	11.95
32	11.1	15.48	21.03	16.58	19.04	13.91	18.34	17.40
33	5.7 min.	21.78	12.97	14.11	17.89	15.83	19.21	16.96
34	8.7	19.16	14.03	13.05	11.37	12.84	11.55	13.67
35	36.1	23.79	17.81	13.56	17.34	13.28	23.06	18.14
36	79.8	16.53	9.77	9.63	11.80	10.24	16.25	12.37
37	114.4 MAX.	19.34	20.18	9.80	8.31	12.47	10.54	13.44
38	109.6	19.81	16.29	17.49	14.31	15.83	18.72	17.07
Sunspot-precipitation coefficient of correlation (r)		−0.4222**	−0.2780*	−0.1260	−0.1779	−0.3891**	−0.2199	−0.2017

** *1% level of significance.*
* *5% level of significance.*

water-equivalent of snow was calculated on the basis of ten inches of snowfall equalling one inch of rainfall.

The coefficient of correlation *(r)* for each station is given at the bottom of the table. The correlation is negative for all stations (a terrene response, according to DeLury's classification). The values of *r* obtained for Edmonton and Battleford are significant to the 1% level, while that for Calgary reaches the 5% level. These significant values, together with the unanimity of agreement of all stations with respect to the negative correlation, are believed to establish the fact that sunspot periodicity exerts a measurable influence on precipitation in the region.

It is possible that special significance may be attached to the fact that the influence of the sunspot cycle is somewhat greater at the most northerly of the stations, Edmonton and Battleford. The fact that the sunspot influence at Calgary is greater than at other southern stations may be due to its position in the foothills.

These geographical distinctions are considered admissible under the hypothesis that factors such as prevailing air currents, varying from region to region, would differ from region to region in their effectiveness in overcoming the sunspot influence. The sunspot factor must be considered as an all-pervading agency which, at a given time, affords to all regions the same degree

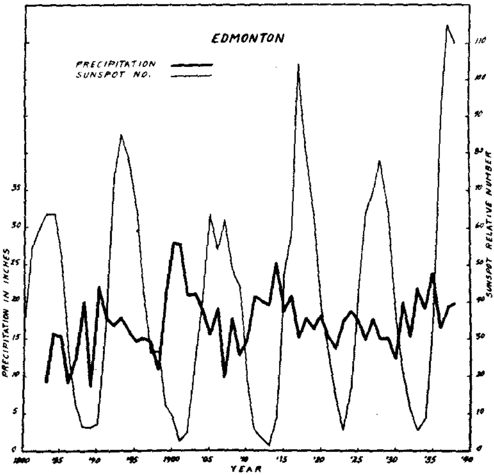

FIG. 2. *Comparison of total annual precipitation at Edmonton with corresponding yearly means for sunspot relative numbers.*

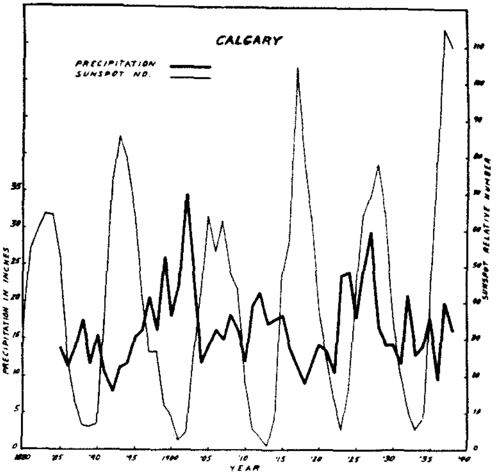

FIG. 3. *Comparison of total annual precipitation at Calgary with corresponding yearly means for sunspot relative numbers.*

of potential influence. The actual influence in a given region is determined by the degree to which the potential influence is affected by the complex of other meteorological factors operating in that particular region.

Graphical representations of the association between sunspot periodicity on the one hand, and precipitation at Edmonton, Calgary, and Battleford on the other, are given in Figs. 2, 3, and 4, respectively. The general tendency for the precipitation curve to rise at sunspot minima and to drop at sunspot maxima (hence minus sign of r values) is reasonably apparent in all graphs. There are, however, a number of breaks in this relation, the most notable being the unusually high precipitation occurring at Calgary in 1927, which was within one year of sunspot maximum. These breaks may be explained by the hypothesis given in the preceding paragraph.

The Calgary graph (Fig. 3) illustrates what may be an important point in connection with the double cycle. It may be significant to note that there have been three periods of excessive drought which centre upon the years 1892, 1918, and 1936, and that each of these drought centres is within one year of a sunspot major maximum. This relation, however, does not hold generally for the other districts under study.

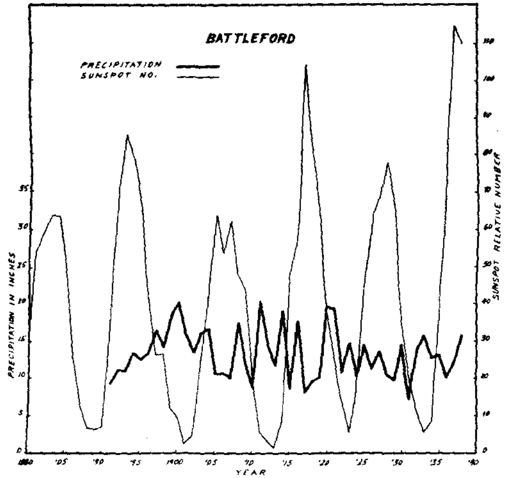

FIG. 4. *Comparison of total annual precipitation at Battleford with corresponding yearly means for sunspot relative numbers.*

(c) *Temperature–sunspot correlations*

In Table II yearly mean sunspot relative numbers are compared with data on mean annual temperature from meteorological stations situated at Calgary, Edmonton, Medicine Hat, Swift Current, Battleford, and Qu'Appelle.

The coefficient of correlation (r) for each station is given at the bottom of the table. In no case is the value of r significant. Some significance, however, may be attached to the fact that, as in sunspot-precipitation correlations, all values of r are negative (terrene response). Irrespective of the significance of individual values of r, as tested by the probable errors based on the populations of individual groups of data, the fact that six consecutive values have shown the same directional relation between the variables may be considered to indicate a possibility that true minus correlation exists, but that the degree of association is not sufficient to be definitely established on the basis of the relatively small period of time for which data are available.

It is concluded, therefore, that while the available data are insufficient to establish a mathematically significant correlation between variations in sunspot number and temperature, there are, nevertheless indications that some association between these variables does exist.

TABLE II

YEARLY MEAN SUNSPOT RELATIVE NUMBERS COMPARED WITH TEMPERATURE DATA FROM SIX POINTS IN ALBERTA AND SASKATCHEWAN

Year	Sunspot relative number	Mean annual temperature in °F. at						Mean of all points
		Edmonton	Calgary	Medicine Hat	Swift Current	Battleford	Qu'Appelle	
1883	63.7 max.	32.9	—	—	—	—	—	—
84	63.5	33.6	35.4	40.1	—	—	30.6	—
85	52.2	36.6	40.0	41.9	—	—	33.2	—
86	25.4	34.5	37.9	41.3	38.4	—	33.8	—
87	13.1	31.5	34.3	37.0	34.5	—	30.9	—
88	6.8	34.7	36.7	39.4	36.0	—	32.4	—
89	6.3 min.	40.9	40.5	43.1	40.2	—	37.9	—
1890	7.1	36.6	37.0	40.1	37.5	—	34.0	—
91	35.6	38.4	38.8	42.0	38.8	32.9	34.8	37.6
92	73.0	36.3	38.1	40.9	37.6	32.6	33.2	36.5
93	84.9 MAX.	34.6	35.3	38.0	35.2	30.3	31.0	34.1
94	78.0	36.8	38.8	43.0	39.7	34.5	36.1	38.2
95	64.0	37.0	37.2	40.0	36.8	33.6	33.4	36.3
96	41.8	34.8	35.9	40.0	37.7	32.5	33.0	35.7
97	26.2	36.7	36.9	40.0	38.0	34.0	34.0	36.6
98	26.7	38.2	37.8	40.9	37.9	34.0	34.2	37.2
99	12.1	34.6	34.7	39.0	35.5	32.4	32.1	34.7
1900	9.5	37.8	38.6	43.6	40.8	34.9	36.4	38.7
01	2.7 min.	39.0	39.2	43.3	40.2	36.5	36.3	39.1
02	5.0	36.9	36.9	41.5	38.4	34.3	35.7	37.3
03	24.4	37.6	37.6	41.9	38.1	33.9	35.0	37.4
04	42.0	36.2	36.9	42.0	37.5	33.4	34.0	36.7
05	63.5 max.	39.4	39.0	43.7	40.1	37.1	37.0	39.4
06	53.8	40.1	39.3	44.0	40.3	36.8	37.2	39.6
07	62.0	35.2	36.7	40.7	35.8	32.2	32.9	35.6
08	48.5	Incomplete	40.7	44.7	39.7	35.2	36.2	—
09	43.9	33.8	36.0	41.1	36.0	32.2	32.9	35.3
1910	18.6	38.4	40.4	Incomplete	41.5	36.6	36.4	—
11	5.7	36.0	35.7	Incomplete	35.9	32.6	32.5	—
12	3.8	39.5	39.4	43.0	38.1	36.6	34.2	38.0
13	1.4 min.	37.8	40.1	43.7	39.0	36.8	35.2	38.8
14	9.6	36.7	40.5	44.3	39.8	37.5	36.5	39.2
15	47.4	39.2	41.8	45.0	40.4	38.6	37.6	40.4
16	57.1	34.8	37.4	39.5	34.9	33.3	32.3	35.4
17	103.9 MAX.	34.6	37.9	41.0	36.1	33.6	33.0	36.0
18	80.6	37.9	41.5	44.1	40.4	36.9	37.4	39.7
19	63.6	35.5	39.5	43.0	38.9	35.5	35.2	37.9
1920	27.6	35.9	38.9	43.2	39.9	36.0	36.4	38.4
21	26.1	37.1	39.7	44.4	40.9	36.7	37.7	39.4
22	14.2	36.7	39.0	42.6	38.3	36.8	36.0	38.2
23	5.8 min.	38.7	40.9	44.7	34.4	38.0	35.0	38.6
24	16.7	36.5	38.6	43.1	38.1	36.1	35.1	37.9
25	44.3	35.9	39.5	43.1	39.4	35.6	36.0	38.3
26	63.9	37.7	41.0	44.8	40.0	36.8	35.7	39.3
27	69.0	33.1	35.7	39.9	37.0	32.7	33.0	35.2
28	77.8 max.	39.0	41.4	44.6	41.8	38.1	37.4	40.4
29	65.0	36.3	38.5	41.7	38.0	34.9	33.8	37.2
1930	35.7	38.7	40.1	44.9	41.1	37.7	36.6	39.9
31	21.2	40.0	41.7	45.3	44.3	39.5	40.9	42.0
32	11.1	36.0	37.6	40.9	38.9	33.3	35.2	37.0
33	5.7 min.	35.2	37.4	41.8	42.8	33.5	35.6	37.7
34	8.7	38.4	41.8	44.9	42.6	37.2	38.4	40.6
35	36.1	37.1	37.1	40.0	37.8	32.2	34.0	36.4
36	79.8	36.1	37.4	40.2	37.5	33.0	33.5	36.3
37	114.4 MAX.	36.9	36.9	40.4	38.3	32.5	35.2	36.7
38	109.6	38.5	40.2	43.0	41.0	36.6	37.7	39.5
Sunspot-temperature coefficient of correlation (r)		−0.1786	−0.0179	−0.0829	−0.0618	−0.2323	−0.0987	−0.1946

(d) Crop yield–sunspot correlations

The demonstration of a mathematically significant causal relation between sunspots and annual precipitation coupled with the determining influence of precipitation (particularly during the growing season) on yield, leads, as a matter of course, to the testing of possible correlations between sunspots and crop yield.

In Table III yearly mean sunspot relative numbers are compared with data on average yields per acre of wheat, oats, and barley in Alberta and Saskatchewan, respectively.

In the case of wheat yield data from both Alberta and Saskatchewan, an attempt was made to include only data from the strictly semi-arid regions (where precipitation is a highly determining factor in yield). In Table III, therefore, the following conditions apply to wheat yield data:

The figures for Alberta represent the total provincial production for the years 1898–1920, inclusive, while for the years 1921–1938, inclusive, they represent only the production for crop districts 1 to 7 inclusive.

The figures for Saskatchewan represent total provincial production for the years 1898–1903, inclusive; while for the years 1904–1938, inclusive, they cover the region represented in 1904–05 by crop districts 1–6; in 1906–07 by crop districts 1–6, 8, 10–13, and 17–20; in 1908–15 by crop districts 1–3, 5, and 6; and in 1916–38 by crop districts 1–4, 6, and 7. [For details on crop districts see Seventh Census of Canada, 1931, vol. VIII (Agriculture)].

Wheat data for Alberta involve only spring wheat, while for Saskatchewan spring and winter wheat data are combined as one figure. The proportion of winter wheat would, however, be negligible.

Data for oats and barley in both provinces represent total provincial production.

The coefficient of correlation (r) for each crop in each province is given at the bottom of Table III. In no case is the value of r significant; but in seven out of eight cases the value is a minus one, and the exceptional plus value is of a far lower order than the minus values generally. This is taken to indicate that the influence of sunspot periodicity on precipitation may carry over in a degree to the secondary result, crop yield.

General Discussion

It is concluded in general that the present study has served to demonstrate that sunspot periodicity has a mathematically measurable influence on precipitation, and possibly also on temperature and crop yields, in certain semi-arid regions of Alberta and Saskatchewan. The points that remain to be discussed are first, the relative importance of the sunspot cycle as one of the factors determining weather, and second, the degree to which the sunspot factor may be used in long-range weather forecasting.

The sunspot-precipitation correlations may be considered as a reasonably conclusive proof of the fact that sunspot periodicity is one of the factors

TABLE III

YEARLY MEAN SUNSPOT RELATIVE NUMBERS COMPARED WITH AVERAGE ANNUAL YIELDS OF WHEAT, OATS, AND BARLEY IN ALBERTA AND SASKATCHEWAN

Year	Sunspot relative number	Mean yield in bushels per acre					
		Wheat		Oats		Barley	
		Alberta	Sask.	Alberta	Sask.	Alberta	Sask.
1898	26.7	25.3	17.3	44.5	24.0	32.0	21.8
99	12.1	23.7	18.4	42.2	30.1	26.8	20.9
1900	9.5	19.2	9.0	33.8	16.6	25.4	18.1
01	2.7 min.	24.6	25.4	40.7	44.7	32.8	31.4
02	5.0	18.9	22.5	31.7	30.9	21.3	20.9
03	24.4	18.7	19.4	32.0	32.7	25.5	24.9
04	42.0	16.6	17.6	31.0	31.0	26.1	24.2
05	63.5 max.	21.5	20.4	39.2	42.7	27.4	27.1
06	53.8	23.1	21.1	39.1	37.4	29.3	24.5
07	62.0	18.3	13.4	30.1	29.0	19.8	17.9
08	48.5	18.8	13.7	36.9	27.2	25.0	17.2
09	43.9	19.0	21.6	35.8	47.1	30.7	32.1
1910	18.6	12.9	15.0	24.7	30.4	20.8	24.5
11	5.7	20.8	18.3	41.2	45.0	29.4	28.0
12	3.8	18.2	20.0	38.2	44.4	27.9	31.1
13	1.4 min.	19.5	19.2	36.1	41.7	25.9	30.2
14	9.6	15.3	12.1	30.2	23.8	23.0	17.9
15	47.4	31.1	25.1	56.7	45.9	34.1	33.2
16	57.1	26.6	13.8	43.8	39.1	28.6	26.5
17	103.9 MAX.	19.0	13.7	32.1	27.2	22.6	21.0
18	80.6	7.7	9.0	22.8	21.5	16.5	17.0
19	63.6	12.0	7.7	28.2	23.1	25.5	18.2
1920	37.6	20.5	10.4	37.3	27.7	26.5	20.2
21	26.1	11.0	12.8	30.0	32.7	22.3	25.9
22	14.2	11.1	20.1	21.5	35.2	14.9	29.0
23	5.8 min.	27.5	20.8	50.0	44.5	38.5	30.0
24	16.7	9.1	10.4	30.0	19.7	25.0	18.2
25	44.3	17.0	17.6	31.0	34.5	25.0	25.4
26	63.9	17.9	15.7	30.0	28.1	22.0	25.1
27	69.0	27.8	19.2	45.0	32.3	30.0	29.3
28	77.8 max.	26.2	23.9	37.7	35.8	29.1	27.3
29	65.0	10.9	9.9	21.9	16.2	17.8	13.8
1930	35.7	17.1	12.2	36.0	27.7	25.4	20.1
31	21.2	13.3	6.1	36.7	15.8	29.3	10.4
32	11.1	19.3	11.9	37.5	24.6	28.1	17.6
33	5.7 min.	9.6	6.0	25.3	16.5	20.3	14.3
34	8.7	12.0	5.7	26.7	13.9	20.1	11.4
35	36.1	12.5	10.1	26.5	26.7	17.8	20.2
36	79.8	5.6	6.5	19.7	14.0	17.0	12.8
37	114.4 MAX.	7.7	0.9	27.6	5.1	22.2	4.7
38	109.6	17.5	8.7	35.0	21.6	26.0	16.6
Sunspot-yield coefficient of correlation (r)	1898–1920	−0.1555	—				
	1898–1903	—	−0.2733	−0.0487	−0.2773	−0.1876	−0.2778
	1921–1938	+0.0165	—				
	1904–1938	—	−0.1943				

determining the amount of precipitation falling in the region under study. Yet the data show that the influence of the factor is often completely nullified and that the correlation in certain regions falls below mathematical significance. The breakdown of the sunspot influence occurs irregularly at any stage of the cycle and may occur at a given time in one region without being in evidence in a similar neighbouring region. These conditions appear to be characteristic of the data and may, therefore, be of some use in formulating a hypothesis on the sunspot cycle as an effective factor in weather determination.

We have some basis, therefore, for stating a hypothesis that presumes the sunspot factor to be a radial agency which pervades uniformly the upper terrestrial atmosphere, but which does not uniformly influence weather components (precipitation, temperature, etc.), since its effectiveness in the lower atmosphere is affected in various ways and to different degrees by a variable complex of meteorological and physical factors. Many of these factors, and we may here include the effective sunspot influence, tend to fluctuate independently in their effectiveness as weather-determining agents. Thus, at different times, different combinations of factors determine the weather. Assuming that certain factors tend to neutralize the effect of the sunspot factor while certain others are indifferent or beneficial, it follows that at different times, as different factors rise and fall in effectiveness, the sunspot factor will fluctuate in power from a nonentity to its fullest expression as a weather-determining agent.

This may be acceptable as a plausible explanation of the irregular breakdown of the sunspot influence on precipitation, but it does not give an answer to the question why, in the region being studied, the data from the two most northerly stations, Edmonton and Battleford, should give considerably higher correlations, in the case of both precipitation and temperature, than do the data from the four southern stations. One might reasonably suppose that the two northern stations are subjected to some relatively stable factor, such as a prevailing air current, which year after year tends to provide conditions conducive to increased effectiveness of the sunspot factor. This point indicates that, in studying sunspot-weather relations, it may be important to treat the data from each station separately.

In the light of the above discussion and within the limits of the present study, what may be said regarding the relative importance of the sunspot cycle among weather-determining factors? It may be said that, in the region under study, sunspot periodicity probably bears some causal relation to precipitation and perhaps also to temperature; but the influence of other agencies tends to suppress and obscure its effect to a point where it becomes in general only a minor contributing factor.

What, then, may be said for the sunspot cycle as a basis of long-range weather forecasting for the region under study? In the first place, long-range weather prediction as a general field is not yet acceptable as a workable science. Before it can be so accepted, we must formulate and prove a theory

of extra-seasonal climatic sequence. Such a theory is not in immediate prospect: first, many meteorological and physical factors, many solar and terrestrial cycles, the overtones of the sunspot cycle itself, must be recognized, disentangled, studied separately, and mastered. What we know at present of the sunspot cycle and its influence on weather is but one step, and a small one, toward an ultimate theory of long-range climatic sequence—and what we know must not be used for more than it is worth.

Acknowledgments

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CONTRIBUTIONS TO A STUDY OF THE FUNGUS FLORA OF NOVA SCOTIA

IV*. ADDITIONAL BASIDIOMYCETES[1]

By L. E. Wehmeyer[2]

Introduction

In previous papers, collections of fungi from Nova Scotia belonging to the Heterobasidiomycetes, Dacryomycetaceae, and Clavariaceae (28), resupinate Hydnaceae (29), and Agaricaceae and Boletaceae (26) have been listed and discussed. The remaining Basidiomycetes are here considered. The large majority of these collections were made during the summer of 1931, with a moderately heavy rainfall, and the summer of 1933, with a very slight precipitation. These collections represent probably only a fraction of the species obtainable over a longer period of time. In 1931, the species of the genus *Hydnellum* were particularly abundant in the more open, moist, conifer forests, and this opportunity was taken to obtain full field notes on the fresh plants in all stages of development.

As indicated in a previous paper (28), all localities cited are in Colchester County unless otherwise designated. The numbers after each collection are the field collection numbers. Species not previously reported from the Province are indicated by an asterisk (*). Colour names given in quotation are those of Ridgeway's *Color Standards* and *Color Nomenclature*.

BASIDIOMYCETES

Exobasidiaceae

***Exobasidium Vaccinii** (Fck.) Wor. On *Vaccinium* sp., Victoria Park, Truro, June 26, 1933 (1540).

Thelephoraceae

Most of the writer's collections of the Thelephoraceae were critically examined by M. A. Donk, who made many of the determinations, as indicated. Most of the species of Hypochnus (Tomentella) were determined by V. Litschauer. For the sake of consistency, Burt's generic separations are adhered to in the following list. Where the determiner's nomenclature differs from this it is indicated in parenthesis.

***Hypochnus cervinus** Burt. On *Poria* sp., Upper Brookside, Aug. 24, 1931, det. V. Litschauer (*Tomentella cervina* (Burt) Bourd. & Galz.) (1404). This determination seems somewhat doubtful to the writer, inasmuch as the colour of this specimen is "vinaceous-drab" to "purple-drab" rather than "fawn colour" as given by Burt for *H. cervinus*. It

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* *The preceding papers of this series were published in the Papers of the Michigan Academy of Science, Arts and Letters (see References 26, 28, and 29).*

is similar to *H. subrinosus* Burt, except that it is not 250–300 μ thick and does not have coloured hyphae near the substratum. This specimen forms a very thin floccose-pruinose coat on the pores of the host. There is scarcely any trama, but the margin thins out to a pale arachnoid subiculum. Clamp connections are doubtfully present. The spores are subglobose to angular, rather coarsely echinulate, hyaline at first, becoming yellow-brown, and are 4–6 × 4–5.5 μ.

* — **fumosus** Fr. On decayed wood, Salmon River region, July 3, 1931 (368); Folleigh Lake, July 20, 1931 (1100); Upper Brookside, July 24, 1931, *fide* M. A. Donk (*Corticium fumosum* Fr.) (1146); Mt. Thom, Aug. 10, 1931 (1252).

* — **fuscus** Fr. On Betula, Victoria Park, Truro, Aug. 30, 1933, det. V. Litschauer (*Tomentella fusca* (Fr.) Schroet.) (1643).

* — **olivascens** (Berk. & Curt.) Burt. On decayed wood, Upper Brookside, Aug. 24, 1931, *fide* V. Litschauer (*Tomentella olivascens* (Berk. & Curt.) Bourd. & Galz.) (1402).

* — **subfuscus** Karst. On Fagus, Upper Brookside, Aug. 11, 1931, det. V. Litschauer (*Tomentella subfusca* (Karst.) v. Höhn. & Litsch.) (1271).

* — ***testaceus*** (Bourd. & Galz.) comb. nov. On *Polyporus versicolor*, Upper Brookside, Aug. 24, 1931, det. V. Litschauer (*Tomentella testacea* Bourd. & Galz.) (1398). The spores (3–5.5 × 5–6.5 μ) and the basidia (8–12 × 2.5—3.5 μ) of this collection are definitely smaller than those given by Bourdot and Galzin for *T. testacea* (6, p. 493). A very fine, floccose-pulverulent, brick-red growth is formed on the pore surface. There is a sparse basal mycelium of dark-brown hyphae, which have clamp connections and are 2.5–6.5 μ in diameter. The hymenium is of compacted hyaline upright basidia. I have seen on description which fits this exactly.

* — **umbrinus** (Fr.) Quél. On beech log, Upper Brookside, Aug. 11, 1931 (1269); on Poria, Upper Brookside, Aug. 24, 1931, det. V. Litschauer (*Tomentella umbrina* (Fr.) Litsch.) (1401). This species is deep blue-black to indigo when fresh, becoming greyish- to brown-cinereous and pruinose on drying. It forms a separable velvety-cottony mat 300–600 μ thick. There is a basal layer of large, loose, dark-brown hyphae, 3–5.5 μ in diameter, without clamps, and an upper layer of lighter coloured, narrower, more closely branched and more compact hyphae, which may have a greenish- to bluish-black tinge and which bear the basidia, which are about 50 × 8–10 μ. In the subhymenial and hymenial layers there are numerous, clavate, gloeocystidial organs, which are transparent and indigo to blue-black in colour. The spores are globose to subglobose, dark brown, coarsely tuberculate-echinulate and 6.5–10 × 6–8 μ.

* **Corticium bombycinum** (Sommerf.) Bres. On *Salix* sp., Victoria Park, July 23, 1931, *fide* M. A. Donk (1121).

* — **botryosum** Bres. On conifer bark, Upper Brookside, July 8, 1931, det. M. A. Donk (*Botryobasidium botryosum* (Bres.) Donk) (428). Burt (10, p. 295) gives this as a synonym of *C. vagum*. The spores of this plant (5–8.3 × 3.3–5 μ, amygdaloid) are smaller than those described by Burt, but this collection fits very well Overholts' (22, p. 509) conception of *C. vagum*. There seems to be little difference between this species and *C. coronatum*, according to Bourdot and Galzin, and our collection is more or less intermediate, but the substrate is similar to that given for *C. botryosum* (limbs of Pine).

* — **calceum** var. **glebulosum** Fr. On fir wood, Salmon River region, Aug. 19, 1933, det. M. A. Donk (1634). In his remarks, Donk states, "Note small cystidia. The specimen agrees with one in the Herb. Fries at Uppsala, as *C. calceum* var. *glebulosum*, from Femsjo, and indicated as the type of the species (*calceum*). Burt, however, had no clear idea of this species. He confused at least three different species: 1—the real *C. calceum* Fr. em. Burt; 2—*C. Romellii* Litsch. in herb.; and 3—*C. pseudo-calceum* Donk in herb., Litsch. in litt. The real *calceum* is rare in Europe, beside the type I know of only one more, from Sweden, collected by Nannfeldt. It seems, however, to be more common in the United States (Burt, Brown, etc.)."

These "cystidia" are tapered toward the apex, or somewhat swollen at the base, and have a more or less swollen globose apex which is often encrusted with crystals that are mostly soluble in potassium hydroxide. They project 15–35 μ beyond the hymenium, and the swollen apex is 3–5 μ in diameter (Fig. 1, c and d).

— **investiens** (Schw.) Bres. (?). On *Rhododendron canadense*, Upper Brookside, July 8, 1931 (433). This species has been transferred to the genera Vararia Karst. and Asterostromella Höhn. & Litsch. by many writers on account of the dichotomously branched, coloured, spinelike, wide-angled branching hyphae or "dichophysen". Donk, upon examination of these plants, considered that it might represent a new species of Vararia, but was unable to find spores. Upon further examination, the writer has been able to locate a few basidia and spores (Fig. 1, *a* and *b*). These spores agree very well with those of *C. investiens*, and the other differences, such as the somewhat lighter and more yellowish coloration, somewhat thinner and less tuberculate fruiting layers, and slightly paler hyphae are largely differences of degree. It seems doubtful if this is more than a variety of *C. investiens*.

The plants are "cartridge buff" to "chamois" when fresh, form elongated effuse patches, and become somewhat paler on drying. The surface is somewhat mealy and friable in character; the margin is sharp and undifferentiated. Hyphae 1.5–2.5 μ in diameter, with wide angled branching and tapering to a point at the ultimate tips. Fruit bodies 400–600 μ thick. Basidia (Fig. 1, *a*) 9–10 × 3–5 μ, four-spored, scattered irregularly over the surface. Spores ellipsoid to fusoid or tapered towards one end, hyaline, 5–9 × 2–3.5 μ (Fig. 1, *b*).

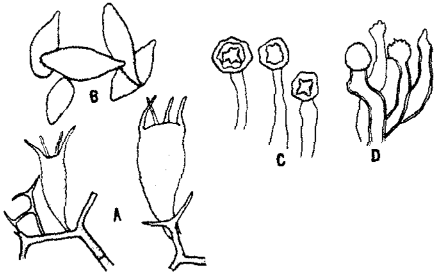

FIG. 1. *A and B. Corticium investiens (Schw.) Bres. (?) (A, basidia, B, basidiospores). C and D. Corticium calceum var. glebulosum Fr. (C, cystidia in water; D, cystidia after treatment with potassium hydroxide).*

* — **leucoxanthum** Bres. On *Acer saccharum*, Upper Brookside, July 15, 1929, det. M. A. Donk (*Gloeocystidiellum leucoxanthum* (Bres.) Donk) (58). This species is apparently new to North America, as pointed out by Donk. The gloeocystidia are merely large, irregular, hyaline hyphae in the hymenium and are easily overlooked, but the amyloid character of the spores is striking.

* — **lividum** Pers. On Betula (?) and Abies, Victoria Park, Aug. 30, 1933, det. M. A. Donk (1642 & 1642a). Waxy-fleshy when fresh and with the margin bluish-glaucous or "citron yellow" to "strontian yellow", often with yellowish strigose or cottony hyphae. Hymenium "vinaceous-fawn", "wood brown", "fawn" or "vinaceous-tawny". When dry, the surface is often thickly covered with small sulphur-yellow granules composed of crystals that break through the surface. Spores ellipsoid, more or less curved or flattened, 3.5 × 1.5–2 μ.

* — **ochraceum** Fr. On decorticated conifer, Mt. Thom, Aug. 15, 1931, det. M. A. Donk (*Gloeocystidium ochraceum* (Fr.) Litsch.) (1338). Considered by Donk as "a very rare species". The gloeocystidia are scarcely distinguishable. They are given by Bourdot and Galzin (6, p. 266) as "non émergentes". There are emergent cystidial elements in this material, however, which are cylindric to conic, 2–4 μ in diameter and projecting 8–16 μ beyond the hymenial surface. These fruit bodies are stratified, and the cystidia may be the early outgrowth of hyphae that begin the formation of a new layer.

* **Peniophora aurantiaca** Bres. On Alnus, Victoria Park, Sept. 7, 1929 (285); Salmon River region, Aug. 25, 1931, *fide* M. A. Donk (1409). Neither Rea nor Bourdot and Galzin mention gloeocystidia for this species. The hymenium is made up largely of swollen hyphal elements, 20–50 × 6–10 μ, which are filled with a granular protoplasm when young. These are as obviously gloeocystidia as those that are found in many species of Gloeocystidium; and it seems probable that many so-called gloeocystidia are merely the young sterile elements of the hymenium, which probably grade off into the differentiated forms in other species.

* — **carnosa** Burt. On *Abies balsamea*, Salmon River region, July 3, 1931 (369); on Fagus, Upper Brookside, July 17, 1931 (1072); on decayed wood, Killag Mines, Halifax Co., July 30, 1931 (1188); Salmon River region, Aug. 19, 1933, *fide* M. A. Donk (1651). Of the many closely similar yellow species of Peniophora, these collections fit *P. carnosa* best. Although not mentioned in Burt's description, the greenish-yellow coloration of the basal hyphae is often due to a granular encrustation. This colour is slowly turned to green and then bleached by 7% potassium hydroxide.

* — **cinerea** (Fr.) Cke. On *Betula alba*, Upper Brookside, July 8, 1931, det. M. A. Donk (430).

* — **cremea** Bres. On *Polyporus betulina*, Upper Brookside, June 27, 1931, det. M. A. Donk (301). This fits *P. cremea* very well except that the spores are somewhat smaller (4.3–6 × 2.5 μ; only a few seen) and that the cystidia protrude only 20–30 μ. Donk considers it either *P. cremea* or *P. parilis* Bourd. (in litt.) but wishes to make further comparisons.

* — **hydnoides** Cke. & Mass. On Fagus, Upper Brookside, July 7, 1931, *fide* M. A. Donk (421). Donk (in litt.) states that all material he has seen from America "differs from the type by the somewhat shorter, broader, and more conical cystidia with thicker wall (in the average), and by the arrangement of the cystidia: being sessile on one or a few axial cystidia-like hyphae in the teeth, more or less enclosed by the trama of the teeth." This he recognizes as a var. *americana.*

This species is placed in the genus Odontia by many writers as *O. hydnoides* (Cke. & Mass.) v. Höhn. This collection shows only extremely minute teeth and is therefore retained in Peniophora. Collections have been made in Michigan that are identical in every respect, except that the teeth are definite and up to 0.5 mm. in length. When fresh, this species has a characteristic bluish-grey translucent appearance. As the teeth enlarge and mature they turn cream colour upon drying, but the margin or context, if exposed, may retain the bluish-grey colour. Most descriptions give the colour as whitish or cream colour.

* — **incarnata** (Fr.) Cke. On Acer, Economy Lake, June 16, 1926, det. M. A. Donk (93). The writer agrees with Donk in his statement (in litt.) that "the hyphae are in some parts of the fructification slightly encrusted. For me (and Bourdot too), *P. affinis* Burt is a synonym of this species". (This collection was first determined as *P. affinis*, by the writer.)

* — **laevis** (Fr.) Burt. On Fagus, Upper Brookside, Sept. 1931, det. M. A. Donk (1514).

* — **pallidula** Bres. On conifer wood, Victoria Park, Truro, Aug. 30, 1933, det. M. A. Donk (1645). This species, which forms a greyish to olive-grey, sparse, floccose growth on decorticated wood, has characteristic cystidia consisting of upright, irregularly cylindric, hyaline hyphae, 3.5 μ in diameter, which have an apical, and often several axial, spherical, light-brown, resinous masses, which are soluble in iodine-chloral-hydrate solution or Amann's mounting medium (lactic acid). The spores were somewhat smaller (3.5 ×

2–2.5 μ) than given by Bourdot and Galzin, and the cystidia were not noticeably nodose, except for the resinous masses.

* — **setigera** (Fr.) Bres. On Abies, Victoria Park, Truro, Aug. 8, 1929 (215); on *Betula alba*, Upper Brookside, June 29, 1931 (325); on Alnus, Upper Brookside, June 27, 1931, det. M. A. Donk (501).

* — **velutina** (Fr.) Cke. On Fagus, Upper Brookside, July 11, 1931 (493); July 28, 1931, det. M. A. Donk (1176). Donk, in his notes, says "*P. velutina*, the form which Burt calls *P. coccineo-fulva* (Schw.) Burt. I do not believe this to be a species different from *velutina*. Bourdot is of the same opinion, according to specimens from his herbarium or named by him". This material is also very similar to that named by Donk as *P. laevis*. That collection (No. 1514) differs only in the somewhat paler colours of the dry hymenium. This paler colour is the distinction used by Burt to separate *P. velutina* and *P. coccineo-fulva*. Burt states (9, p. 257) that *P. laevis* differs from *P. coccineo-fulva* in the narrower basal hyphae. Bourdot (6, p. 307) gives the basal hyphae of *P. laevis* as 7–8 μ and those of *P. velutina* as 8–10 μ. In all these collections the hyphae vary from 4 to 9 μ. There are a number of other species in this same group that are difficult to distinguish, and it is apparent that entirely too many species have been erected with no regard to already described ones.

***Aleurodiscus amorphus** (Fr.) Rabenh. On *Abies balsamea*, Upper Brookside, June 29, 1931 and July 7, 1931 (331 & 331b); Middle River, Victoria Co., Cape Breton Island, Aug. 5, 1931 (331a). Common on dead or dying standing fir, the pinkish-orange fruiting bodies resembling a Discomycete.

* — **scutellatus** Litsch.† On bark of spruce, Upper Brookside, July 26, 1929 (77); on standing conifer, Salmon River region, July 14, 1931, det. M. A. Donk & V. Litschauer (77a). As pointed out by Donk, this species has been reported only from two localities in Europe (Northern Alps and Hohe Tatra) and from the Chinese province of Yunnan. It is new to North America. These plants are small, 1–4 mm. in diameter, more or less circular and with upturned margins, giving them a cup-shaped appearance. The outer surface is light sand-colour, and the hymenium greyish-pulverulent. The fruit body is 300–400 μ thick and stratose. The hymenium contains numerous large basidia, 10–13 μ in diameter and up to 60 μ in length, and smaller sterile hyphae, 3–4.5 μ in diameter and sometimes nodose-constricted at the apex. Spores large, hyaline, ovoid to subglobose, 12–15 × 10–13 μ.

* **Stereum abietinum** Fr. On fir log, Salmon River region, Aug. 19, 1933, det. M. A. Donk (1635). This species has a superficial resemblance to *S. sanguineolentum* but is almost entirely resupinate and contains large cylindric, thick-walled, dark brown, encrusted cystidia, 5–9 μ in diameter, which project up to 70 μ beyond the hymenium.

— **fasciatum** Schw. On hardwoods, Folleigh Lake, Sept. 23, 1927 (33 & 34), leg. A. R. Prince (6036 & 6153); Salmon River region, July 15, 1931 (1048).

* — **Murrayi** (Berk. & Curt.) Burt. On Acer, Mt. Thom, Aug. 15, 1931, *fide* M. A. Donk; on Betula, Salmon River region, July 14, 1931 (1021); Folleigh Lake, July 20, 1931 (1108); Mt. Thom, Aug. 15, 1931, *fide* M. A. Donk (1319). Burt (8, p. 131) does not mention gloeocystidia for this species, but Bourdot and Galzin (6, p. 381) point out that they are always present in American material. These collections show such clavate to elongate gloeocystidia. They are 25–40 × 6.5–9 μ, and are filled with a yellowish oily substance when fresh. All transitions between these gloeocystidia and the vesicular bodies of the lower tramal layers are found; and it is apparent that the gloeocystidia become swollen and lose their oily content, and so give rise to the vesicular bodies.

— **purpureum** Fr. On *Salix* sp., Bible Hill, Truro, Nov. 21, 1931, coll. A. R. Prince, det. M. A. Donk (1657).

†This species has been shown to be a synonym of A. subcruentatus (Berk. & Curt.) Burt, by M. K. Nobles (Mycologia, 29 : 387. 1937).

* — **roseo-carneum** (Fr.) Schw. On Fagus, Upper Brookside, Oct. 24, 1926, coll. A. R. Prince, det. M. A. Donk (1516); Sept. 3, 1931, det. M. A. Donk (1516a).

* — **rufum** Fr. On *Populus* spp. Upper Brookside, July 13, 1926 and July 19, 1929 (108 & 135). Common on poplar.

— **sanguineolentum** Alb. & Schw. On *Abies balsamea*, Salmon River region, July 15, 1931 (1045); Boularderie Island, Cape Breton Co., Aug. 4, 1931, *fide* M. A. Donk; Salmon River region, Aug. 13, 1931, *fide* M. A. Donk (1300); Mt. Thom, Aug. 15, 1931 (1331). Common on dead standing fir. The coloured conducting organs are not always conspicuous in these fruit bodies and often terminate in the hymenium as hyaline, slightly pronged or encrusted, cystidia-like hyphae.

* **Hymenochaete badio-ferruginea** (Mont.) Lév. On Populus, Upper Brookside, June 29, 1931 (344); on Acer, New Glasgow Road, June 30, 1931 (345); on Abies, Earltown Road, Aug. 22, 1931 (344a). This is probably merely a form of *H. tabacina* upon upright stems, but as these collections are quite distinct, they are grouped here under this species name.

* — **corrugata** (Fr.) Lév. On Fagus, Upper Brookside, Sept. 28, 1926, coll. A. R. Prince (109); on Acer, Upper Brookside, June 29, 1931 (317); on *Betula alba*, July 1, 1931, *fide* M. A. Donk (359).

* — **corticolor** Berk. & Rav. On Fagus, Upper Brookside, July 16, 1929, *fide* M. A. Donk (50 & 51). No. 50 has the appearance of *H. corrugata*, being almost entirely effuse on the under side of limbs and cracked into small areas. It has a much lighter (bistre) colour, however, has a distinct, sharp, blackened margin, and is stratose. Donk has suggested that it is a form of *H. corticolor* (on the under side of limbs) which is undoubtedly true, although it is lighter in colour, more cracked, and effuse.

* — **spreta** Pk. On dead hardwoods, Folleigh Lake, Sept. 3, 1928, *fide* M. A. Donk (21), coll. A. R. Prince (6085); Upper Brookside, July 4, 1931 (398); July 8, 1931 (444); July 17, 1931 (1073).

— **tabacina** Lév. On *Acer spicatum*, Upper Brookside, Sept. 3, 1926 (111); Earltown Road, Aug. 22, 1931 (346a); on *Acer pennsylvanicum*, Upper Brookside, Aug. 12, 1931 (1290); on *Betula alba*, New Glasgow Road, June 30, 1931 (346); on *Taxus canadensis*, Salmon River region, July 14, 1931 (1022); on *Salix* sp. Victoria Park, Truro, July 23, 1931 (1127). Common on a large variety of hosts.

Cyphella fasciculata (Berk. & Curt.) Schw. On Alnus, Upper Brookside, July 13, 1931 (1006); Salmon River region, Aug. 25, 1931, *fide* M. A. Donk (1408).

* **Solenia anomala**† (Pers.) Fck. On Fagus, Upper Brookside, Aug. 3, 1929 (210).

* — **fasciculata**† Fr. On Betula, Upper Brookside, Aug. 3, 1929 (213); Salmon River region, Aug. 1, 1931 (1202); Aug. 13, 1921, *fide* M. A. Donk as *Henningsiomyces fasciculatus* (Pers.) Donk (1202a). This species was quite common on decaying birch trunks, forming confluent areas of fruiting bodies, usually on the surface of the bark cortex beneath the periderm.

Craterellus cornucopioides Fr. On soil under hardwoods, Upper Brookside, Aug. 26, 1931, coll. A. H. Smith (1414).

* — **infundibuliformis** Fr. Under conifers, Victoria Park, Truro, Aug. 23, 1929 (259).

* — **pistillaris** Fr. On soil in mixed woods, Upper Brookside, July 22, 1929 (214); Aug. 31, 1931, coll. A. H. Smith (214a). There has been much discussion as to whether this species is distinct from *Clavaria pistillaris*. The collections placed here are rather distinct, in the much broader and more or less depressed apex, from those (No. 1417 & 1417a) placed

† Donk, in a letter, has pointed out that the genus name Solenia has several homonyms (Solena Lour. and Solena Willd. in the Phanerogams, Solenia Agardh. in the algae, and Solenia J. Hill in the Boletaceae). He is proposing the name Henningsiomyces O. Kze. for Solenia fasciculata and its relatives, and Cyphellopsis for S. anomala and related species. The species are listed here under Solenia merely to avoid confusion.

under Clavaria. Young stages were followed in No. 214. The younger fruit bodies (0.5–6 cm. tall) were cylindric to obtusely conic, covered with a yellowish pruinosity, solid and white within and with a pale orange coloration towards the apex. Later, the upper portion of the club became longitudinally rugose or wrinkled and canary-yellow. The apex then became truncate-depressed to disc-shaped and reddish-orange. The oldest plants were 6–9 cm. tall and 1–2 cm. across the apex. Basidia, but no spores, had developed, the hymenium developing first on the ridges and later in the furrows of the wrinkled stipe. The plants of No. 214a were 6–8 × 2–3 cm. and turbinately expanded at the apex into a flattened pileus. The spores were 10–11 × 5–5.5 μ.

* **Thelephora anthocephala** Fr. var. **clavularis** Quél. On moss and duff, Earltown Road, Aug. 22, 1931, det. M. A. Donk (1389).

* — **multipartita** Schw. Under hardwoods, Earltown Road, Aug. 21, 1931, *fide* M. A. Donk (1382). This collection contains some specimens in which the margin is only slightly divided by shallow splits and which would agree perfectly with Burt's conception of *T. regularis*. Burt's statement (7, p. 206) that these two species are doubtfully distinct and that this should be a variety of *T. regularis* is probably correct.

* — **palmata** Fr. Under conifers, Salmon River region, July 15, 1931 (1047). Odour of radish or garlic when fresh.

— **terrestris** Fr. On soil and duff, usually under conifers, Glenholme, July 16, 1927 (23), coll. A. R. Prince (6037); Upper Brookside, July 3, 1931 (275); Salmon River region, Aug. 1, 1931 (1195); Killag Mines, Halifax Co., July 30, 1931 (1185); Salmon River region, Aug. 13, 1931, det. M. A. Donk (1294, 1295, 1301). Donk says of these collections "1195 is probably an undeveloped specimen of *T. intybacea* Fr., but I am not sure about that. European mycologists of more recent date do not recognize *terrestris* and *intybacea* sensu Fries as different species. I found in Sweden abundant specimens of the latter but am not prepared to say whether they are different or not. At this moment my impression is that the two are connected by many intermediates". No. 1195 consists of erect cylindric masses with practically no expanded pileate portions.

Hydnaceae (stipitate forms)†

Hericium coralloides (Scop.) Pers. On Fagus, Wentworth Valley, Cumberland Co., Aug. 29, 1931, coll. A. H. Smith (1427). This specimen approaches *H. caput-ursi*, having very massive main branches and a massive base, but with the branches up to 8 cm. long and much divided. The teeth are pure white, up to 15 mm. long, and clustered in fascicles on the terminal branches.

* — **laciniatum** (Leers) Banker. On Fagus, Mt. Thom, Aug. 15, 1931, coll. A. H. Smith (1310); Earltown Road, Aug. 21, 1931, coll. A. H. Smith (1310a). Base 2 cm. broad, 4 cm. high, white with a pinkish cast. Primary branches stout, 1–1.5 cm. in diameter, covered with teeth. Secondary branches short (0.5–1 cm.) with long teeth (1–3 mm.).

* **Hydnellum cyaneotinctum** (Pk.) Banker. Under conifers, Upper Brookside, July 28, 1931 (1172); Aug. 31, 1931, coll. A. H. Smith (1172b); Victoria Park, Truro, Aug. 17, 1931 (1172a); Salmon River region, Aug. 13, 1931 (1292). Occurring singly, gregarious, or sometimes confluent. Pileus 4–11 cm. in diameter, obconic, sometimes flat or convex, but usually somewhat depressed, surface usually uneven or strongly colliculose, spongy-pubescent, soft-spongy when dry, white to pinkish-cinnamon or red-brown when fresh ("light vinaceous-cinnamon" to "vinaceous-cinnamon" or "salmon-buff") with bluish areolations when young, which rapidly fade but may persist as bluish tints on stem, teeth, or context. A reddish juice is exuded when fresh, which turns the plant yellow-brown to blackish where bruised and dries a pale tan or black in these areas. Margin obtuse, sterile, often bluish. Stem central, rather elongate, rooting, 2–10 cm. long,

† *An account of the resupinate Hydnaceae has been given in a previous paper by Wehmeyer and Cejp (29).*

tapering downward, 2 cm. in diameter above, 2–6 mm. below, spongy-tough, covered with a white to bluish spongy tomentum that turns bluish where bruised, or in age, only occasionally bulbous at the base as given by Banker. Teeth cylindric, crowded, 2–3 mm. long, shorter toward the margin, decurrent, pinkish-white at first, soon with a definite, bluish, glaucous bloom ("Ontario violet"), finally becoming reddish-brown from the base upwards, black where bruised, drying a pale cinnamon or black. Context duplex, surface spongy, interior woody-fibrous, somewhat zonate, pinkish-tan with dark watery zones or bluish, sometimes greenish, areas, often bluish along the margin, drying pinkish-tan to dark brown. Spores irregularly subglobose, coarsely tuberculate, light yellowish-brown, 4–5 × 3.5–4.5 μ. Odour slightly fragrant, taste mild.

This species is quite common under conifers. Although it has the cinnamon or reddish-brown colours, in age, of many other species of the genus, when fresh it has the whitish tomentum with bluish areas similar to that found in *H. suaveolens*. It differs from this latter species in the depressed cap, uneven surface of the pileus, less fragrant and less persistent odour, and the lack of the deep indigo coloration of the stem context.

* — **diabolus** Banker. Under conifers, Salmon River region, July 15, 1931 (1039); Aug. 1, 1931 (1173a) Aug. 13, 1931 (1173b); Upper Brookside, July 28, 1931 (1173). This species is usually gregarious, sometimes complicate. Pilei stout obconic, expanding with age, 2–12 cm. broad, convex or only slightly depressed, surface smooth, even or slightly undulate, covered with a dense cottony tomentum. The very young plants are stout obconic and white, soon showing pinkish to cinnamon spots that exude a bright red juice. As they mature, the plants become variegated with salmon, reddish, red-brown, or blackish areas (vinaceous-cinnamon, "tawny", "madder brown", "garnet brown", "maroon" or "black"), turning dark red-brown to black where bruised. Margin obtuse at first, thinning out at maturity. Stem central to lateral, sometimes short, stout, usually elongate, rooting in the moss or duff, 1–7 × 0.5–2.5 cm., covered with a tomentum that is at first white but soon turns cinnamon-brown or black where bruised. Teeth terete, up to 3 mm. long, shorter towards the margin, somewhat decurrent, "light Congo pink" to "Japan rose" at first, becoming red-brown to black. Context duplex, surface layers spongy, interior tougher, radiately fibrous, pinkish-cinnamon to red-brown, zonate. Spores subglobose, rather dark yellow-brown, tuberculate, 2.5–5.5(6) μ in diameter. Odour woody-fragrant when fresh. Taste tardily acrid. Plants drying a dull reddish-brown with surface tomentum somewhat strigose under the lens.

This species is also quite abundant under conifers and can be distinguished by the reddish juice and reddish colorations of the pileus. The plants described by Coker (11) under this name have a more salmon coloration and a colourless juice.

— **floriforme** (Schaeff.) Banker. Under conifers, Economy Lake, Aug. 31, 1927 (67), coll. A. R. Prince (6230); Salmon River region, July 15, 1931, coll. A. H. Smith (1040); Victoria Park, Truro, Aug. 17, 1931 (1040a). Plants variable, characterized by the presence of orange-coloured tomentum upon the pileus or at least upon the stipe. Colour variable, young plants usually pure white, becoming light orange. In some plants the orange coloration is retained, even after drying. In others the tomentum turns darker, becoming orange-brown to olive-brown ("light salmon-orange" or "bittersweet pink" to "orange-chrome", "dragon's-blood red") or almost black. Teeth white at first, becoming vinaceous-to olive-brown, shorter towards margin, not decurrent on stem tomentum. Context tough-fibrous with a cottony soft outer layer, dull yellow- to olive-brown or orange-brown, somewhat zonate. Spores oblong to spherical, tuberculate, pale yellow-brown, 4–5.5(6) × 2.5–5 μ. Odour, woody-fragrant; taste, farinaceous.

As figured by Schaeffer (24) and accepted by Banker (2) in his description, *H. floriforme* includes a wide range of forms. Two rather distinct groups were recognizable, however, in the Nova Scotian collections, agreeing with the *H. compactum* Pers. and *H. hybridum* Pers. or *H. aurantiacum* (Batsch) Alb. & Schw. as mentioned by Banker (2, p. 160). Collection No. 67 contains plants similar to those collected about Ann Arbor, Mich., and as pictured by Coker (11, Plate 19), which fit very well Schaeffer's

Plates 146, Fig. 4, and 147, Figs. 5 and 6, and Batsch's (4) Plate 11, Figs. *222a*, *b*, and *c* (*H. hybridum* of Persoon or *H. aurantiacum* of Fries). These plants are slighter in stature with thinner margins and more uneven, colliculose, or compounded pilei. They have more depressed or concave surface and are more brilliant orange in their colour, which is retained longer, being present usually in the dried plant. The remainder of the Nova Scotian collections are represented well by Figs. 1–3, 5, and 6 of Schaeffer's Plate 146 (*H. compactum*). These plants are pure white at first, stouter, obconic with convex surfaces and thicker margins. The young plants are cottony tomentose at first and soon take on deeper orange-brown, olive-brown, or red-brown shades. In the older plants of this group, the colorations are duller and present only in local areas of the pileus or, more commonly, the stipe. Sufficient collections have not been made to determine whether these two groups are distinct, and as most writers consider them variations of one species, they are here united. It would be interesting to determine, however, whether *H. compactum* is a more northerly form, as seems possible.

* — **geogenium** (Fr.) Banker. Under conifers, Upper Brookside, Aug. 28, 1931 (1419). Plant squat, 4–7 cm. across; stem short, appearing almost sessile, inconspicuous. Pileus complicate, usually with a number of more or less fused stems arising from a common mass of olive-brown to black tomentum from which extends the greenish-yellow mycelium which is the key colour to the plant. Pilei irregularly circular, around the central stem mass, olive-black in the centre shading to dark olive-brown with areas of light yellow-brown or white towards the margin (probably lighter in young plants), somewhat depressed, radiate fibrillose, appressed tomentose, surface roughened, rugose to scrobiculate, complicate, often with smaller pilei arising from the surface; margin thin, often imbricate with such pilei. Substance watery-spongy, somewhat fibrous, dark olive-green or with yellow-brown areas. Individual stems small, up to 1 cm. long and 2–4 mm. in diameter, olive-brown, fused together with a mass of tomentum 1–3 cm. across. Teeth cinereous to vinaceous-brown, somewhat glaucous, tips bright greenish-yellow ("citron yellow" to "pale lemon yellow"), up to 3 mm. in length, only slightly shortened towards margin, decurrent on stem. Spores subglobose to oblong, pale-yellowish, tuberculate, $3.5–4.5 \times 2.5–3.5\ \mu$. Odour slightly woody-fragrant.

This collection agrees perfectly with Fries' (14, p. 9, Plate 8) description and figures. Banker (2, p. 135), in 1906, recorded a collection of this species from New York by Peck, stating he considered the determination "not wholly satisfactory". Later (3), he states that this collection is typical of the species as found in Europe. This Nova Scotian collection, therefore, stands as an interesting link between the European range and its single previous occurrence in eastern North America.

* — **mirabile** Fr. On needle duff under conifers. Victoria Park, Truro, July 23, 1931 (1118). Plants very squat, appearing almost sessile or buried in the duff on account of a short stipe, obconic to almost hemispheric, centrally stipitate. Pileus rather bright yellow-brown, turning darker as the light, yellowish, watery juice exudes over the surface and turning lighter again upon drying. Surface covered with a thick, dense tufted, strigose-tomentose upper layer, convex, fairly even or irregularly roughened. Margin thick, rounded, glabrous, lighter in colour except where the juice is pressed out, and then darker, hygrophanous. Stem short, stout, $1–2.5 \times 1–4$ cm., mostly buried in the duff, often appearing confluent, but pilei mostly regular, sometimes with a brownish tomentose covering. Teeth short and stout towards the margin, longer cylindric-pointed towards stem, 3 mm. long, light brown with lighter coloured tips, decurrent on stem. Substance watery-tough, upper portion spongy-hairy, giving a duplex context, dark brown from juice, hygrophanous, zonate. Spores brown, tuberculate, spherical, 4–5 μ in diameter. Taste rather acrid, odour slightly fragrant.

These plants are characterized by their stout, squat, convex build, their light-brown colour, their densely strigose-tomentose surface and very watery-hygrophanous character, the yellow juice staining the flesh dark brown wherever bruised. The only plant that seems to approach these characters is *Hydnum mirabile* Fr. Fries' figure (14, Plate 3,

Fig. 2) of this species shows a plant with similar build and strigose tomentosity, but the colour is too light and too yellowish for our plants. It may be that the colour turns darker with age or that these plates do not express the colour accurately. In 1896, Peck (23, p. 112) reported what he considered as *H. mirabile* from New York. He gave the odour as farinaceous and the taste as similar but becoming peppery. He remarked especially upon the duplex character of the context, which set it off from *H. acre* of Quélet. He gave the colour as greyish-buff to brownish-buff. Lloyd (18, Letter 49, p. 12; 51, p. 4; 53, pp. 9, 11; 58, p. 5) states that *H. mirabile* is rarer in Europe than in eastern America and that Peck's plants are this species and the same as a species collected by Atkinson and named by Bresadola as *H. cristatum* (1, p. 119), but differs from *H. acre* Quél. in the surface of the pileus and the softer context. Atkinson (1, p. 119) gives the pileus as "ochre-yellow, covered with numerous strigose hairs in the form of crests". Our plants agree with these American descriptions of *H. mirabile*.

— **parvum** Banker. On spruce duff, Salmon River region, Aug. 13, 1931, coll. A. H. Smith (1296). This is the plant first described as *H. zonatum* (Batsch) Karst. by Banker (2), but later (3) found to be distinct from the European species. This collection agrees in all respects with Banker's description, except that the teeth are sometimes longer (up to 2 mm.) and somewhat decurrent, and that the spores are larger (4–5 × 3.5–4 μ).

* — **suaveolens** (Scop.) Karst. Under fir and spruce, Salmon River region, July 14, 1931, coll. A. H. Smith (1035); Aug. 1, 1931, coll. A. H. Smith (1035a); Victoria Park, Truro, Aug. 17, 1931 (1035b); Upper Brookside, Aug. 28, 1931, coll. A. H. Smith (1035c). Plant rather stout and squat, 4–15 cm. in diameter. Pileus obconic, thick, convex or plane, surface rather even or slightly undulate, white at first, covered with a thick velvety tomentum, turning watery grey to light olive-brown where bruised. Margin thick, sterile. Stem usually short and stout, 2–4 cm. long, 1.5–2 cm. thick, covered with a spongy tomentum which shows shades of bright blue ("pale blue-violet" to "dark dull violet-blue") or patches of white. Teeth terete, short, blunt at first, becoming long and slender (up to 6 mm.), shorter towards margin, somewhat decurrent, whitish at first, then glaucous bluish and finally brownish from the base upwards. Substance duplex, upper layer soft spongy, white, inner portions tough, radiate-fibrous, drying woody, somewhat zonate, whitish above, shading into a deep indigo-blue below and in the stem. Spores small, subglobose, hyaline to light yellowish, tuberculate, 3.5–5 × 3–4 μ. Odour strongly fragrant, long persistent, even in dried plants. Taste none or mealy.

This northern species was abundant under fir and spruce and is easily distinguishable after one becomes acquainted with the plants in various stages. The smooth, convex, white, velvety surface, bluish tomentum on the stipe, bluish teeth, strongly fragrant odour and deep indigo coloration of the substance of the stem are all good diagnostic characters that are retained in the dried plants.

* — **zonatum** (Batsch) Karst. Under conifers and hardwoods, Princeport (65), coll. A. R. Prince (6227); Killag Mines, Halifax Co., July 30, 1931 (1181). This is the species formerly referred to by Banker (2) as *H. concrescens* (Pers.) Bank. The plants from Killag Mines are not particularly zonate but are more radiately rugose or scrobiculate. They may be *H. scrobiculatum* or *H. hybridum*, but until better series are obtainable they should remain here.

Hydnum repandum L. On soil under conifers, Upper Brookside, July 31, 1931, coll. A. H. Smith (1193); Wentworth Valley, Cumberland Co., Aug. 29, 1931 (1193a); Princeport, Aug. 20, 1927 (57), coll. A. R. Prince (6225).

* **Phellodon coriaceo-membranaceus** (Schw.) Banker. Under spruce, Upper Brookside, Aug. 14, 1931, coll. A. H. Smith (1303); Economy River, Aug. 31, 1927, coll. A. R. Prince (6231). Banker (2) gives this species as differing from *P. tomentosus* in the larger pilei which are less confluent and in the stronger odour. The collections referred here, which have larger pilei (1.5–7 cm.) and are less confluent than those referred to *P. tomentosus*, had a slightly fragrant odour, but this odour was actually less strong than that of the

small plants referred to *P. tomentosus*. It seems doubtful whether this odour is a constant character.

— **tomentosus** (L.) Banker. Under spruce and fir, Victoria Park, July 23, 1931, coll. A. H. Smith (1122); Salmon River region, Aug. 18, 1931 (1122a). The plants referred to this species were smaller (1.5–3 cm.), more densely confluent, and possessed a pungent, asafoetida-like odour, which is retained in the dried plants, in contrast to the slightly fragrant odour of the *P. coriaceo-membranaceus* plants.

* — **vellereus** (Pk.) Banker. Under hardwoods, Folleigh Lake, Aug. 29, 1931, coll. A. H. Smith (1430). Although these plants have a sweetish, putrid, asafoetida-like odour when fresh, similar to that given for *P. putidus*, they are smaller than that species and agree more closely with the descriptions of *P. vellereus*.

* **Sarcodon radicatus** Banker. On soil under upturned stump, conifer woods, Portapique Beach, Aug. 7, 1933 (1615).

* **Sarcodon stereosarcinon** (†) *sp. nov.* (Plate I). On soil under conifers, Victoria Park, Truro, Aug. 23, 1929 (262); Upper Brookside, July 4, 1931 (380); Aug. 1931 (380a). Pilei 3–12 cm. in diameter, centrally stipitate, lobed or complicated with several pilei springing from a common stalk, rather thick-fleshy-fibrous, tan to tawny, pale cinnamon- or reddish-brown ("vinaceous-cinnamon"), glabrous, minutely velvety-tomentose or cracking with age and then appearing somewhat scaly. Margin irregular to lobed, thin, light coloured beneath, sterile. Stipe central, eccentric or lateral, 2–10 cm. long, 1–2 cm. in diameter, sinuous or compound, tapering downwards, dirty-brown, smooth, solid, drying hard and woody. Teeth crowded, slender, terete or flattened, short and whitish at the margin, elongating rapidly and becoming dull brown from the base upwards, the light tips giving a cinereous-brown sheen in mass, strongly decurrent on the stem, sometimes falling away and then leaving small pits with an intervening raised reticulum. Context radiating fleshy-fibrous, whitish, or darker in stem, drying hard and brittle. Spores pale brown in mass, hyaline under the microscope, globose to ovoid, coarsely tuberculate, 3.5–5 × 2.5–4 µ. Odour strongly farinaceous.

This Sarcodon, which is intermediate between this genus and Hydnellum, is the most abundant in the province. It is common in moist seasons in open coniferous woods, occurring in large troops. Its disposition is a difficult one. In a previous paper (28), the writer referred to these collections as *Sarcodon montanus* on the basis of a specimen collected by Kauffman at Lake Cushman, Washington, and labelled *Hydnum montanum* (in Herb., Univ. of Michigan). Further examination has thrown doubt upon the identity of these plants, particularly in the absence of notes on the fresh condition of Kauffman's plant. Kauffman's plant has very thin flesh and a stout stem which is stuffed with a spongy reticulate mass of dried tissue, whereas the Nova Scotia plants have a relative thick, firm, white flesh and a solid stipe which dries firm and woody. Kauffman's material was sent to Lloyd who replied as follows "A fleshy plant with hyaline tuberculate spores, rare characters and only present in *H. fragile* (equals *H. reticulatum*, letter 54, note 224) and this is surely not this plant; probably unnamed". Lloyd's note 224 refers to a collection made by Miss Hibbard in Nova Scotia and referred by Lloyd to *H. reticulatum*.

† **Sarcodon stereosarcinon** *sp. nov. Pilei 3–12 cm. diametro, stipite centrali, lobati complicative aliquot stipite unico adscendentes, aliquantum crassi-carnoso-fibrati, fusci, fulvi, pallido-cinnamomini vel rubro-brunnei, (vinaceo-cinnamomini Ridgewayi), glabri minute velutino-tomentosi vel vetustate dehiscentes deinque aliquantulum squamosi visi. Margo irregularis vel lobatus, tenuis, infra pallidus, sterilis. Stipes centralis, excentricus vel lateralis, 2–10 cm. longus, 1–2 cm. diametro, sinuosus vel compositus, ad basim attenuatus, sordide fuscus, glaber, solidus, rigidus lignosusque exsiccatus. Dentes conferti, gracilis, teretes vel complanati, margine breves et albidi, velociter producentes et e basi sursum obscuro-fusci, apices lucidi cumulo nitorem cinereo-fuscum parientes, admodum in stipitem decurrentes, nonnunquam cadentes denique lacunas pusillas reticulum interiectum elevatumque creantes. Contextus albidus, stipite minors albido, carnoso-fibratus confluens rigidus fragilisque exsiccatus. Massa sporarum pallido-fusca. Sporae translucidae, globosae usque ad ovatae, crasse tuberculatae, 3.5–5 × 2.5–4 µ. Odor farinaceus.*

PLATE I. *Sarcodon stereosarcinon sp. nov.*

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Miss Hibbard's plants "grew in troops", had hyaline tuberculate spores, dried wood-brown with paler flesh and had flattened decurrent teeth which left a reticulum when broken off. All these characters, as well as Lloyd's photograph (18, Letter 56, Fig. 715) agree perfectly with our own Nova Scotia collections, and there is little doubt that they are the same species.

This is not Banker's *Sarcodon reticulatus*, however, as is shown by an examination of the type distribution (Ellis, N. Am. Fung. 929). *S. reticulatus* is a lighter colour and intermediate in thickness of flesh. The reticulations in this species, from which the specific name is taken, are the flattened and confluent bases of the teeth, forming definite raised ridges. Reticulations can be found in *Sarcodon stereosarcinon* also, and were noted as a "minor feature" by Lloyd. These reticulations are of a different sort, however, and are caused by the presence of a sunken, circular pit or depression where the older teeth fall away from the pileus, the ridges being the intervening anastomosing reticulum of pileus tissue.

All three of these species are similar in general appearance and in the hyaline tuberculate spores, but *Sarcodon stereosarcinon* differs in the stouter, thicker build, firmer, more fibrous flesh, and somewhat darker coloration. The plants also tend to be more complicate and in this condition resemble closely Fries' figures of *Hydnum versipellis* (14, Plate I), but are a more tawny- or cinereous-brown and are not so definitely scaly.

Polyporaceae

The collections of Poria were examined by D. V. Baxter, and unless otherwise indicated the determinations of this genus are his. The writer is also indebted to J. L. Lowe for the determination of a number of species of Polyporus and for helpful suggestions as to the disposition of others.

Porothelium fimbriatum Fr. On Betula, Salmon River region, July 14, 1931 (1016); New Glasgow Road, July 25, 1931 (1151); on Fagus, Upper Brookside, Aug. 11, 1931 (1278); on Populus, Upper Brookside, Aug. 11, 1931 (1279). Quite common, reported from the Province as *P. Friesii*.

Merulius lacrymans Fr. On conifer cord wood, at the base of a moist pile in a shed, Truro, Aug. 30, 1933, *fide* M. A. Donk (1639).

* — **porinoides** Fr. On Fagus, Upper Brookside, July 1, 1933, det. M. A. Donk (1570). Donk remarks "as far as I know, new to the American continent". This species is very similar to *M. crispatus*, *M. serpens*, and *M. ceracellus*. The last species is an American one. This collection has smaller pores (3 per mm.) and somewhat smaller spores (2.5–3.5 × 2 μ) than are given for *M. porinoides*. On the other hand, it is thicker (500–800 μ), has somewhat larger pores than *M. ceracellus*, and has clamp connections on the hyphae, which Burt states are not present in that species.

The fruit bodies are widely effuse, somewhat separable, white at first, and at the margins, then "massicot yellow" and with a smooth surface, which is later pushed up into irregular anastomosing ridges which form the pore surface which is darker brown ("reed yellow" to "olive-buff").

* **Poria ambigua** Bres. Inside of stump, Upper Brookside, Sept. 4, 1931 (1470); on Populus, Victoria Park, Truro, July 18, 1933 (1586).

* — **attenuata** (Pk.) Cke. On Fagus, Upper Brookside, July 30, 1929 (188); Aug. 11, 1931 (1270); on Salix, Victoria Park, Truro, July 23, 1931 (1119); on Betula, Middle River, Victoria Co., Aug. 5, 1931 (1232). This species is considered the same as *P. eupora* Karst., *P. Blytii* Fr., and a number of other species by Baxter.

* — **corticola** (Fr.) Cke. On Populus (?), Upper Brookside, June 28, 1931 (314).

* — **ferrea** (Pers.) Romell. On Alnus, Victoria Park, Truro, July 18, 1933 (1592). This species is known under the name of *Polyporus ferreus*, but is resupinate and is better in the genus Poria.

— **ferruginosa** (Schrad.) Fr. On Fagus, Upper Brookside, June 29, 1931 (327). Baxter states that no spores were found in this material and that the determination could not be certain, but the plant belongs in the *P. ferruginosa* group.

— **punctata** Fr. On Salix, Victoria Park, Truro, July 23, 1931 (1126).

— **semitincta** (Pk.) Cke. On decayed wood, Salmon River region, Aug. 1, 1931 (1206); on Betula, Mt. Thom, Aug. 10, 1931 (1246).

— **subacida** (Pk.) Sacc. On conifer logs, Killag Mines, Halifax Co., July 30, 1931 (1186); Mt. Thom, Aug. 15, 1931 (1316); Victoria Park, Truro, Aug. 15, 1933 (1627).

— **subincarnata** (Pk.) Murr. On conifer log, Upper Brookside, July 11, 1931 (491); on Abies, Middle River, Victoria Co., Aug. 5, 1931 (1231).

— **vulgaris** Fr. sensu stricto ex Romell non Bresadola. On Abies, New Glasgow Road, June 30, 1931 (338).

— **xantha** Lind. On Populus, Salmon River, Aug. 13, 1931 (1298).

Poria sp. On *Abies balsamea*, Victoria Park, Truro, Aug. 8, 1929 (234). Baxter states that this collection represents the type of plant which has been called *Poria flavicans* Karst. in America. A study of many American collections and a recent comparison with European material has convinced him, however, that these American collections are not the *P. flavicans* of Karsten, but represent a new species which he hopes to describe later.

Polyporus abietinus Fr. On *Abies balsamea*, Victoria Park, Truro, Aug. 8, 1929 (222). Although often considered as merely a growth or host form of *Polyporus pargamenus*, these effuse reflexed forms on coniferous wood are definitely distinct from the large dimidiate or imbricate, more yellowish pilei common on hardwoods. This collection shows the irpicoid or lamellate condition of the pores, the hymenophore consisting strictly not of pores at all, but branched lacerate lamellae that radiate out from numerous centres of growth. This condition is the *Irpex fusco-violaceus* of Fries, the lamellate condition being even more pronounced here than in the figure shown by Lloyd (17, p. 1230).

— **adustus** Fr. On Populus, Victoria Park, Truro, Oct. 3, 1925, coll. A. R. Prince (88); Upper Brookside, July 27, 1931 (1163).

— **albellus** Pk. On Betula, Folleigh Lake, Aug. 13, 1927, coll. A. R. Prince (6041); on *Acer saccharum*, Upper Brookside, Aug. 1, 1929 (197); on Fagus, Folleigh Lake, July 20, 1931 (1091); Salmon River region, July 15, 1931 (1043). Common on hardwoods. Inasmuch as *P. albellus* seems to be the only name definitely fixed for the species complex including such names as *P. trabeus* Fr., *P. chioneus* Fr., and *P. lacteus* Fr., and inasmuch as various writers are unable to agree whether these species are distinct and what characters should be used for distinction, all the collections falling in this group are placed here.

* — **anceps** Pk. On Pine (?), Salt Springs, Pictou Co., Sept. 2, 1925, coll. A. R. Prince (42). This plant fits the conception of this species given by Kauffman (15) rather than that of the western plants that turn sordid red with age, mentioned by Lloyd (17, p. 655) and Kauffman. In this plant the margin becomes yellowish-brown, the pores, context, and most of the surface remaining pure white.

— **balsameus** Pk. On *Abies balsamea*, Upper Brookside, Aug. 14, 1931 (1308); Sept. 3, 1931, coll. A. H. Smith (1308a). These plants were found on the roots and bases of fir and were determined by J. L. Lowe. They were sessile, dimidiate, imbricate, 2–7 $\times$ 1–3 $\times$ 0.2–0.5 cm., watery, tough-fibrous, and zonate with shades of yellow-brown, greyish-brown and greyish-white. The surface was radiately fibrous-tomentose and the margins obtuse, sterile, often white-tomentose and somewhat undulate. Context watery-white, 1–2 mm. thick; tubes 0.5–4 mm. long, mouths white, round, or sometimes sinuous or almost daedaloid, 3–4 per mm. Spores ellipsoid, 3.5–4 $\times$ 1.5–2.5 μ, hyaline. Odour disagreeable.

— **benzoinus** Fr. (*P. fuscus* Pers., *P. resinosus* Fr.). On *Tsuga canadensis*, Upper Brookside, Sept. 3, 1931, coll. A. H. Smith (1468). This may be merely a form of *P. resinosus* as held by Overholts (21, p. 47) and others, but these specimens fit so perfectly the concept of *P. benzoinus* recognized by Lloyd (17, p. 333, 334) on conifer hosts that they are placed here under that name. The surfaces of the pilei are dark chocolate-brown, definitely hispid and with bluish-black zonate bands, obvious even in the dried plants. The pore mouths are pale yellowish-white at first, the rest of the pore being brown. Later the pore mouths turn darker or blackish where bruised and remain firm in drying. The plants have a pungent fragrant odour when fresh.

— **betulinus** Fr. On Betula, West River, Salt Springs, Pictou Co., Sept. 2, 1925, coll. A. R. Prince (83). Very common throughout the Province on birch.

— **borealis** Fr. On living roots of spruce, Victoria Park, Truro, Aug. 17, 1931 (1324); Sept. 1, 1931, coll. A. H. Smith, det. J. L. Lowe (1324a). These plants are somewhat thinner (0.5–1 cm.) than is given in most descriptions and often formed erect, circular, fasciculate clusters about a central point of attachment. Spores ellipsoid, apiculate, light yellow-brown in mass, 5–5.5 × 3.5 μ. Odour sweetish. Taste slightly nauseating.

— **caesius** Fr. On beech (?), Salmon River region, Sept. 7, 1931, det. J. L. Lowe (1481).

— (*Trametes*) **cinnabarinus** Fr. Economy Lake, June 17, 1926; on Fagus, Folleigh Lake, Aug. 23, 1927 (87); on Betula, Mt. Thom, Aug. 11, 1927, coll. A. R. Prince (6161); Folleigh Lake, Aug. 18, 1927, coll. A. R. Prince (6073).

* — **cinnamomeus** Fr. On mossy soil, Lake O'Law, Inverness Co., Aug. 6, 1931 (245a). A small, silky, bright cinnamon coloured plant in contrast to the common *P. perennis*, which is usually found on sandy soil. Although the spores seen in this plant were immature, measuring about 5 × 2.5–3.5 μ, they show the characteristic differences from those of *P. perennis*, as pointed out by Lowe (20, p. 45), that is they are stout ellipsoid, often slightly inequilateral, have no central oil globule, and are somewhat apiculate. The tramal hyphae are thick-walled, unbranched, and 10–12 μ in diameter.

— **circinatus** Fr. On soil under conifers, particularly spruce, Victoria Park, Truro, Aug. 17, 1931, coll. A. H. Smith (1325); Upper Brookside, Aug. 20, 1931, coll. A. H. Smith (1325a); Salmon River region, Aug. 1931.

— **confluens** Fr. On soil at base of Abies, Victoria Park, Truro, July 22 and Aug. 17, 1931, coll. A. H. Smith (1115a, 1115d). In the fresh condition this species is often easily confused with *P. ovinus*, but the differences, especially of the dried plants, mentioned by various authors are shown by these collections also. Number 1115a shows the fasciculate condition with fused stems. In drying, the surface of the pileus becomes yellowish-buff or black where bruised, but always shows more or less of the reddish coloration characteristic of this species. The pores, context, and stem also show these flesh, red-orange, or red-brown tints. Spores ellipsoid, apiculate, with a single oil droplet, 3.5–4.5 × 2–3 μ.

— **elegans** Fr. On decayed sticks, Upper Brookside, July 24, 1929 (112).

* — **fagicola** Murr. On Fagus, Folleigh Lake, July 20, 1931, det. J. L. Lowe (1092); Upper Brookside, July 1, 1933 (1092a). According to Lowe, this species represents a smooth, small-pored form of *P. squamosus* or a small-pored form of *P. pennsylvanicus*, if it can be recognized as a species at all. The cap is smooth to radiately appressed- and scaly-tomentose.

* — **fibrillosus** Karst. On *Abies balsamea*, Mt. Thom, Aug. 10, 1931. det. J. L. Lowe (1249).

* — **fragilis** Fr. On conifer log, Earltown Road, Aug. 21, 1931 (1377); on spruce log, Salmon River region, Sept. 7, 1931, det. J. L. Lowe (1477).

* — **glomeratus** Pk. On a maple log, Mt. Thom, Aug. 10, 1931 (1248). Found effused over wide areas on the under side of the log but becoming reflexed at the margins, which seems to be the characteristic position of this species. This collection shows the typical,

large, dark-brown, seta-like hyphae as pointed out by Lloyd (18, Letter 54, p. 4) for this species. These hyphae are imbedded for the most part in the trama but may project through the hymenium and then appear as setae, which Lloyd (17, p. 1012) later pointed out to be a variable condition. The yellowish spores of this plant vary from globose to broad ellipsoid, 3.5–5.2 × 3–3.5 μ, rather than being globose and 5–6 μ as given in most descriptions. When fresh, the pore surface has a characteristic sheen, being silver-white to grey when the light is reflected from the white pore mouths and greenish- to olive-brown when the light is reflected from the interior of the tubes.

* — **guttulatus** Pk. On conifer stub, Mt. Thom, Aug. 10, 1931 (1247); on Hemlock, Mt. Thom, Aug. 15, 1931, coll. A. H. Smith, det. J. L. Lowe (1321). The spores were ellipsoid, hyaline and 3.5 × 2 μ. Odour slightly fragrant.

* — **hirsutulus** Schw. On *Acer pennsylvanicum*, Upper Brookside, Aug. 12, 1931 (1291). This may be merely a form of *P. versicolor*, but it is lighter in colour, more strongly hirsute, has a yellowish pore surface, and is more effuse-reflexed with small pilei.

— **hirsutus** Fr. On Betula, Economy Lake, June 16, 1926, coll. A. R. Prince (17); on Fagus, Folleigh Lake, July 20, 1931 (1096 & 1097); Salmon River region, Aug. 18, 1931 (1096a).

* — **hirtus** Quél. At base of Abies, Earltown Road, Aug. 26, 1931, det. J. L. Lowe (1115b). Although this plant is centrally stipitate, it fits very well the description given by Shope (25, p. 357). The spores were immature, elongate-ellipsoid with a curved apiculus at one end, hyaline and 9–10 × 3.5 μ. Although fairly common in the west, this species seems to be rather rare in the eastern states.

— **lucidus** Fr. On old stump, Victoria Park, Truro, Aug. 8, 1929 (247). Fairly common and reaching a great size on conifer logs.

* — **nidulans** Fr. On *Betula alba*, New Glasgow Road, July 25, 1931 (1134); on Fagus, July 30, 1931, det. J. L. Lowe (1184)

* — **ovinus** Fr. On soil, Victoria Park, July 22, 1931, coll. A. H. Smith (1115); Upper Brookside, Aug. 31, 1931 (1115c). In contrast to *P. confluens* (see notes under that species), these plants grow singly and are centrally stipitate. Upon drying, the pileus and pore layers are pale yellowish-olive, or blackened where bruised. There is often a pinkish flesh coloration at the base of the stem, but not over the whole plant as in *P. confluens*. When fresh the cap turns bluish very slowly where bruised. The spores of 1115c were broad ellipsoid, apiculate, and 4.5–5 × 3–3.5 μ.

— **pargamenus** Fr. On hardwood stubs and logs, Middle River, Victoria Co., Aug. 5, 1931 (1503); Princeport, Aug. 26, 1927, coll. A. R. Prince (6160). On upright hardwood stubs the pilei of this species are formed in great numbers and reach a size of 10–21 × 6–7 cm.

— **perennis** Fr. On soil (usually sandy), Westcook's Cove, Guysborough Co., Sept. 5, 1925, coll. A. R. Prince (1052); Upper Brookside, June, 1927, coll. A. R. Prince (85); Victoria Park, Truro, Aug. 16, 1929 (245); Salmon River region, Aug. 1931 (1509). In contrast to *P. cinnamomeus*, as pointed out by Lowe (20, p. 44), the spores of these plants are symmetrical, oblong ellipsoid, have a large globule in the centre of each spore, and are 6.5–8 × 3.5–5 μ. The tramal hyphae are 4–6 μ in diameter.

— **picipes** Fr. On buried wood, Mt. Thom, Aug. 11, 1927, coll. A. R. Prince (89); Folleigh Lake, Aug. 23, 1927, coll. A. R. Prince (14).

— **radiatus** Fr. On Betula, Salmon River region, Sept. 7, 1931, det. J. L. Lowe (1248b); on Acer, Upper Brookside, Sept. 4, 1931 (1248a). The setae are quite abundant in these plants. They are dark brown, 9–10 μ in diameter and project 20–25 μ. They are sharply pointed, curved at the apex, and vesciculose at the base. Spores hyaline, subglobose, 3–4 × 2.5–3 μ.

— **Schweinitzii** Fr. On conifer roots, Victoria Park, Truro, Aug. 1929.

* — **semisupinus** Berk. & Curt. On Betula, Folleigh Lake, July 20, 1931 (1505); Upper Brookside, Aug. 11, 1931 (1205); Earltown Road, Aug. 22, 1931 (1285a); on Fagus,

Upper Brookside, Aug. 12, 1931 (1285); Aug. 14, 1931 (1307). Collections determined by J. L. Lowe.

— **sulphureus** Fr. On Betula, Mt. Thom, Aug. 1, 1929 (187).

— **varius** Fr. On Apple, Truro, June 20, 1926, coll. A. R. Prince (6273); July 2, 1929 (216); on Maple stump, Upper Brookside, July 10, 1933 (552a). Specimens determined by J. L. Lowe. The specimens from Apple have all been collected from the same tree, which has produced a crop of sporophores every summer that the writer has visited the Province. The tree is badly decayed and begins to form fruiting bodies late in June. The same fruiting bodies continue to develop throughout the summer. A good deal of variation in colour is encountered with age. The young sporophore is watery, tough, pale cream colour to tawny yellow-brown, with a smooth or finely and minutely silky-striate surface. The pore surface is pale tawny at first and the margin is thin, sterile, and inrolled. The stem is white toward the apex but black velvety towards the base. The pilei turn darker with age, the central disc becoming yellow-brown and the radiating striae reddish-brown. The margin becomes yellowish- to reddish-brown or almost black. The pore surface also becomes darker brown and the context becomes firm and corky, yellow-brown and 5–12 mm. thick. The pilei are up to 10 cm. in diameter, eccentric or laterally stipitate, and more or less depressed at the apex of the stem, especially when young. The margin is irregularly lobed or crenate; the pores are 3–4 per mm., 1–4 mm. long and decurrent on the stem. The spores are ellipsoid, hyaline, usually apiculate, and 6–7.5 × 2.5–3 μ.

These plants are very similar to *P. admirabilis* and *P. Underwoodii*, but they are never pure white as described by Dodge (13) and others for these species. Our plants seem to be more darkly coloured and smaller than Dodge's plants, and with a lesser tendency to be clustered and infundibuliform.

The specimens from Acer are somewhat smaller (4–8 cm. broad; context 2–6 mm. thick) and the surface fibrils do not turn reddish-brown as they do on the plants from Apple.

— **velutinus** Fr. On Fagus, Mt. Thom, Aug. 15, 1931, coll. A. H. Smith, det. J. L. Lowe (1311). These plants fit very well the description given by Kauffman (16, p. 212). They are thicker than *P. versicolor*, are pure white or with greyish to greyish-brown tints when fresh, slightly colliculose or pubescent towards the point of attachment, radiately rugose, glabrous, and zonate toward the thin margin. They dry a pale creamy white.

— **versicolor** Fr. On Fagus, Upper Brookside, July 24, 1929 (101); on decayed logs, Economy Lake, June 16, 1926 and Mt. Thom, Aug. 11, 1927, coll. A. R. Prince (6269 & 6049).

Trametes heteromorpha (Fr.) Lloyd. On conifer, Upper Brookside, 1933 (1655).

— **hispida** Fr. On Populus, Salmon River region, July 3, 1931 (361).

— **Pini** Fr. On Abies, New Glasgow Road, June 30, 1931 (343); on spruce, Salmon River region, Sept. 7, 1931 (1480); Moore's Lake, Halifax Co., July 5, 1929 (33). No. 33 is the widely effuse, annual form with a single, lighter yellow-brown pore layer which is placed by some writers in *Trametes piceinus* Pk. or the variety *Abietis* Karst.

— **subrosea** Weir. On spruce, Upper Brookside, Sept. 3, 1926, coll. A. R. Prince; on fir, New Glasgow Road, June 30, 1931 (339); Victoria Park, Truro, Aug. 17, 1931 (1507).

Fomes applanatus (Wallr.) Gill. On Acer, Salt Springs, Pictou Co., Sept. 3, 1925, coll. A. R. Prince (6267); on living apple tree, Truro, Aug. 18, 1925, coll. A. R. Prince (1053).

— **connatus** (Fr.) Gill. On *Acer saccharum*, Upper Brookside, July 27, 1929 (158).

— **fomentarius** Gill. On Acer, Westcook's Grove, Guysborough Co., Sept. 5, 1925, coll. A. R. Prince (1035); on Betula, Economy Lake, June 16, 1926 (13); on living Fagus, Upper Brookside, June 22, 1926 (57). Abundant on standing beech.

— **igniarius** Gill. On Betula, Westcook's Cove, Guysborough Co., Sept. 1925, coll. A. R. Prince (1046); Salmon River region, Aug. 13, 1931 (1506); on *Pyrus malus*, Truro, June 28,

1936; on Populus, Salmon River region, Aug. 1, 1931 (238a). This species is quite common. No attempt has been made here to separate the numerous varieties and forms that have been described. The plants from Betula are probably the variety *nigricans* although all types of fruiting bodies, from entirely effuse to strongly ungulate, are found. They all have a comparatively smooth upper surface, which is often light coloured. The cystidia are also quite numerous and dense, which suggests the form given by Lloyd (17, p. 247) as *F. borealis*. The resupinate forms are probably those known as *Polyporus leavigatus* or *F. nigricans* var. *laevigatus* Overh. (5, p. 430).

— **pinicola** (Swartz) Cke. On conifer logs, Economy Lake, June 17, 1926 (34); Folleigh Lake, Oct. 22, 1926, coll. A. R. Prince (6255); on living Betula, Upper Brookside, Aug. 8, 1927, coll. A. R. Prince (6258). Common.

* — **scutellatus** (Schw.) Cke. On Alnus, Upper Brookside, June 27, 1931 (305); Salmon River region, July 15, 1931 (1508).

Favolus canadensis Klotzsch. On Fagus, Upper Brookside, June 29, 1925, coll. A. R. Prince (6257); July 21, 1929 (110). Common on beech.

Daedalea confragosa Fr. On Alnus, Salmon River region Aug. 1, 1931 (1209); on Betula, Middle River, Victoria Co., Aug. 5, 1931 (1209a).

— **unicolor** Fr. On Betula, Victoria Park, Truro, Oct. 3, 1926, coll. A. R. Prince (6163); on hardwood log, Upper Brookside, July 14, 1929 (68); on Fagus, Upper Brookside, June 29, 1931 (328); Sept. 11, 1931 (68a). Nos. 68a and 6163 have the surface of the pileus light yellow-brown rather than greyish to greenish and are the *D. ochracea* of Lloyd, according to J. L. Lowe.

Lenzites betulina Fr. On Betula, Economy Lake, June 16, 1926 (5); on Fagus, Folleigh Lake, July 20, 1931 (1093).

— **sepiaria** Fr. On Abies, Moore's Lake Halifax Co., July 5, 1929 (34); Upper Brookside, June 27, 1931 (309); on buried wood, Folleigh Lake, Aug. 23, 1927, coll. A. R. Prince (6164). Common on conifer hosts.

Sclerodermataceae

Scleroderma vulgare Fr. On soil, Upper Brookside, coll. A. R. Prince (6215).

Lycoperdaceae

Bovista pila Berk. and Curt. On soil, Killag Mines, Halifax Co., July 30, 1931 (1215); beach, St. Peters, Richmond Co., Aug. 3, 1931 (1215a); Baddeck, Victoria Co., Aug. 5, 1931 (1224a); Salmon River region, Aug. 18, 1931, coll. A. H. Smith (1370).

— **plumbea** Pers. On soil, Baddeck, Victoria Co., Aug. 5, 1931 (1224); Upper Brookside, Aug. 24, 1931, coll. A. H. Smith (1405).

* **Calvatia elata** (Massee) Morgan. On old sticks, and climbing 6 in. up an old alder trunk to fruit, Upper Brookside, Aug. 14, 1931, coll. A. H. Smith (1302). Fruit body 5–8 × 4–7 cm., having an elongated, obconic stipe, 3–5 cm. long, and a globose flattened head. Outer peridium of minute furfuraceous warts or spines, which are soon deciduous; inner peridium extremely brittle, pale yellowish, becoming olivaceous. Spores globose, brown, coarsely echinulate, 4–5 μ.

Lycoperdon gemmatum Batsch. On soil or humus of decayed logs, Upper Brookside, July 17, 1931 (1076); Folleigh Lake, July 20, 1931 (1076a); Earltown Road, Aug. 22, 1931 (1076b). Very common and quite variable.

— **polymorphum** Vitt. (*L. furfuraceum* Schaeff.)†. In open pasture, Salmon River region, Aug. 18, 1931, coll. A. H. Smith (1369). Fruit body spherical to flattened, 2–3.5 cm., outer periderm of minute floccose warts (4–6 per mm.), creamy white. Chambers of gleba very minute or invisible to the naked eye. Spores globose, brown, smooth, with a large guttula and occasionally a very short pedicel, 3.3–4 μ in diameter. Capillitium of long, narrow, branched threads, about 4μ in diameter and tapering off to fine points, dark brown.

This plant is placed here on account of the unchambered gleba, narrow, dark coloured capillitium and short pedicelled, guttulate spores. The capillitial threads are long and intertwined, but taper off to pointed ends and are rather richly branched, suggesting a Bovistella more than a Lycoperdon. This is the spherical form with small sterile base, referred by Lloyd (17, p. 234) to *L. cepaeforme*. The bright olive-yellow or reddish-yellow colour of the dried plants also suggests the form placed by Coker and Couch (12, p. 93) in *L. coloratum* Pk.

— **pyriforme** Schaeff. On log, Folleigh Lake, Oct. 1928, coll. A. R. Prince (235).

* — **subincarnatum** Pk. On beech log, Upper Brookside, Aug. 12, 1931, coll. A. H. Smith (1286); on decayed wood, Earltown Road, Aug. 19, 1931 (1286a); on sawdust pile, Earltown Road, Aug. 19, 1931 (1286b). A species quite common on woody substrata, characterized by its vinaceous-brown colour ("vinaceous-fawn" to "Mikado brown") and the minute, stout-pyramidal spines of the outer cortex, which are often split, but connivent at the apex. In age the inner peridium shows as a membrane, pock-marked by numerous pits. Spores 3.5–4.5 μ in diameter, very minutely echinulate.

* — **umbrinum** Pers. On soil and humus of decayed logs, Upper Brookside, July 24, 1931, coll. A. H. Smith (1136c); July 24, 1931 (1137); Earltown Road, Aug. 8, 1931 (1136a). A small turbinate species (3–5 cm. diameter), light greyish to yellow-brown in colour (lighter below) with very fine, filiform, somewhat curved cortical spines. Spores globose, brown, guttulate, finely echinulate, 3.5–5 μ in diameter and with a short hyaline pedicel about 1μ in length.

Nidulariaceae

Crucibulum vulgare Tul. On herbaceous stems, Salmon River region, Aug. 1, 1931 (1214); on rotten wood, Economy Lake, July 1926, coll. A. R. Prince (6264).

Sphaerobolaceae

* **Sphaerobolus stellatus** Tode. On beech wood, Upper Brookside, July 1, (353); on cow dung, Upper Brookside, July 1, 1931, coll. A. H. Smith (354); on moose dung, Killag Mines, Halifax Co., July 30, 1931 (1182).

Hysterangiaceae

* **Phallogaster saccatus** Morgan. On soil above birch log, Salmon River region, Aug. 18, 1931 (1249).

Phallaceae

Mutinus caninus Fr. Around fir stump, Folleigh Lake, July 20, 1931, coll. A. H. Smith (1087). This seems to be the European species with slender cylindric stalk, sharply marked but short glebal area, no perforation of the apex, and whitish stem downwards. Coker and Couch (12, p. 10) consider *M. caninus* as probably synonymous with *M. Ravenellii*, but Lloyd, throughout, considers *M. Ravenellii* as a species which may be a form of *M. elegans*, the western American species with stouter stem tapered toward the apex and rosy throughout, a view also accepted by Stomps (27).

Dictyophora duplicata (Bosc.) E. Fischer. On soil, Upper Brookside, Sept. 5, 1931, coll. A. H. Smith (1472).

† *This specimen was sent to Lohwag, who would place it under the name L. furfuraceum Schaeff., for the reasons stated in his notes as follows: "Nach Hollos wird L. furfuraceum Schaeff. und L. pusillum Batsch von Bulliard unter dem Namen cepaeforme verwechselt und ist daher L. cepaeforme zu streichen. Da nach Hollos L. polymorphum Vitt. synonym zu furfuraceum ist, so wäre nach Hollos dieser Pilz mit furfuraceum Schaeff. zu bezeichnen. Ich sehe keinen wesentlichen Unterschied. Auch die sterile Basalpartie ist sehr dicht, was von Hollos als charakteristisch bezeichnet wird. Hollos hat furfuraceum als sehr polymorph bezeichnet."*

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VARIETAL DIFFERENCES IN THE RELATION BETWEEN PROTEIN CONTENT OF WHEAT AND LOAF VOLUME OF BREAD[1]

BY A. G. McCALLA[2]

Abstract

Loaf volume obtained with the malt-phosphate-bromate formula (1 mg. of bromate per 100 gm. of flour) is highly correlated with wheat protein within any one variety of hard red spring wheat. The extremes of protein content studied were 8.0 and 20.0%, and there were few incidents of significant variation from linear correlation.

The regression of loaf volume on protein varied enormously from one variety to another. The regression coefficient is just as much an inherent varietal characteristic as is yield or protein content.

Introduction

The protein content of hard red spring wheat has for some years been considered as the best single measure of flour strength available (1, 8). This is particularly true when comparisons are confined to individual varieties of wheat (3), but high correlation coefficients have also been obtained from loaf volume and protein data involving several varieties (5, 6, 8, 12).

Not all investigators have obtained results in agreement with these conclusions (2, 7, 9, 10), but it seems likely that most of the disagreement can be explained by the baking formulas used by these investigators, since these are not suitable for high protein flours. This is well illustrated by results obtained by Larmour (8) using the basic and bromate formulas. The correlation coefficient (loaf volume – protein) with the basic formula was only 0.630 as compared with 0.903 for the same flours baked by the bromate formula. Similar results have been obtained by Harris (5, 6).

In a recent study, Aitken and Geddes (1) investigated the effect of protein content of flour on the loaf volume of bread. The distinction between this and earlier studies lies in the fact that the protein content was altered by adding prepared gluten to a low protein flour. Thus all samples were basically the same. Correlation coefficients were not given, but it is obvious that these

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were high. The authors concluded that the results yielded linear regressions over the whole range of protein content.

Waldron and Mangels (11) stressed the importance of regression coefficients as distinct from correlation coefficients in evaluating the relation between loaf volume and protein content. This is particularly important when varieties are being compared.

The studies reported in this paper were begun in an effort to explain baking results obtained with a series of wheats that included a large number of very low protein samples. It was found that, while high protein Reward wheat produced very large loaves of bread, low protein wheat of the same variety made bread of much smaller volume and poorer quality than did samples of Red Bobs and Marquis wheat of comparable protein content. This was at first attributed to the source of the material, so an intensive study of results obtained with wheats grown in comparable plots was undertaken.

Material and Methods

Material and data from a number of sources have been used in this study. Results obtained in our own laboratory are confined to those of the crops grown from 1935 to 1938 inclusive. All baking of these samples was done by one technician. The results discussed are grouped into the following series:

1. *Alberta Wheat Quality Surveys*, including results for the varieties Garnet, Marquis, Red Bobs, and Reward. This series was made up of farmers' samples, most of which were collected from machines at the time of threshing. Wheat from all parts of Alberta is included and results are for 1936, 1937, and 1938. In 1936 and 1937, each sample collected was milled and baked, and the correlation analysis was carried out with these individual results. In 1938, a protein determination was made on each sample, and then the samples were composited by variety, protein content, and official variety zones, which are based primarily on soil types. These composites were milled and baked, and the loaf volume results correlated with mean protein values.

In 1935 a preliminary survey was made, but only over a small part of the province. Composites of each variety were made up for each zone involved and the composites baked. Similar composites were made up for each variety and zone in 1936 and 1937. The combined results for the three years are included in the correlation studies of this general series.

2. *Comparative Tests at Northern Alberta Stations*, including the varieties Garnet, Marquis, Red Bobs, and Reward. Wheat was grown at six stations in 1936, seven stations in 1937, and six stations in 1938 in randomized quadruplicate six rod-row plots. Protein determinations were made on individual samples and baking tests on composites of the four replicates.

3. *Fertilizer Tests on Grey Soil*, including Garnet and Reward. Wheat was grown at Fallis in 5 × 5 Latin squares, each plot being six rod-rows in size. Five treatments with ammonium sulphate at various times and rates were used. In each of 1936 and 1937 one square was grown on wheat stubble,

and in 1938 one square on oat stubble and one on newly broken clover sod. Protein determinations were made on wheat from individual plots, and baking tests on composites of the five replicates. Results for the three years are included in the correlation analysis.

4. *Miscellaneous Tests with Reward Wheat.* Reward wheat is used in fertilizer tests carried out by the Department of Soils, University of Alberta, at Breton (grey soil) and in rotation experiments by the Experimental Substation at Beaverlodge. Milling and baking tests of the former, and protein, milling, and baking tests of the latter are made in our laboratory. The results obtained with each of these series have been subjected to a correlation analysis.

5. *Co-operative Tests with Standard and New Varieties* produced at stations over Western Canada for the Sub-committee on Plant Breeding of the Associate Committee on Plant Diseases. This series, made up of samples obtained by compositing wheat from three or four stations, contains standard varieties and all the licensed rust resistant varieties grown in Western Canada. Results for a few unnamed and unlicensed hybrids are also included. The protein results were obtained in the Grain Research Laboratory, Winnipeg, while the loaf volume results are means of those obtained at the Grain Research Laboratory and at the Universities of Saskatchewan and Alberta.

All baking results were obtained using the malt-phosphate-bromate formula (1) with 1 mg. potassium bromate per 100 gm. loaf.

Results

All statistical analyses carried out in this study follow the methods described by Goulden (4).

Studies Involving the Varieties Commonly Grown in Alberta, Series 1 to 4

A summary of the four years' results obtained with the material collected in the Alberta wheat quality survey is given in the first part of Table I. All correlation coefficients are highly significant, but in general the highest values were obtained with the series in which composites were studied. This is accounted for by the fact that peculiarities of individual samples tend to disappear when several samples are composited. This is particularly important with the survey samples, since some of these, although predominantly of the variety listed, contained other varieties as impurities. Thus a sample of Garnet carrying 10% of Red Bobs or Marquis wheat produced bread of larger volume than comparable pure Garnet.

The most important feature of these results, however, is the variability in the values of the regression coefficients. There is both a seasonal and varietal variation; the former is accounted for largely in the gradual improvement in the technique of the experimental baker. That the varietal differences are highly significant is shown by the results of an analysis of variance carried out on the regression coefficients obtained from all series in which these four varieties were included. These results are given in Table II. Based on

TABLE I

RESULTS OF UNIVERSITY OF ALBERTA TESTS ON THE VARIETIES COMMONLY GROWN IN ALBERTA

Variety	Year	No. of samples	Mean protein ($\bar{p}$), %	Mean loaf volume ($\bar{v}$), cc.	Standard deviation of p, %	Standard deviation of v, cc.	r_{vp}	b_{vp}, cc.
Series 1. Wheat quality survey								
Garnet	1936	82	10.8	522	1.50	62.4	.830	34.5
Marquis	1936	41	13.2	709	2.35	110.0	.817	38.2
Red Bobs	1936	37	12.7	719	2.11	103.1	.880	43.0
Reward	1936	30	12.8	677	2.06	131.7	.897	57.3
Garnet	1937	119	12.3	596	1.72	90.8	.884	46.8
Marquis	1937	54	13.8	806	1.89	126.9	.926	62.0
Red Bobs	1937	76	12.9	762	1.90	96.4	.897	45.5
Reward	1937	33	13.3	748	1.86	127.1	.878	59.9
Garnet	1938	8	11.9	522	1.23	72.7	.842	49.8
Marquis	1938	12	12.9	726	2.12	131.4	.964	59.9
Red Bobs	1938	14	12.4	721	1.63	94.8	.914	53.2
Reward	1938	10	14.0	772	2.09	174.6	.993	83.1
Garnet	1935–37	9	11.7	549	1.50	67.5	.956	43.2
Marquis	1935–37	11	12.9	721	1.82	95.4	.911	47.8
Red Bobs	1935–37	10	12.4	710	1.71	82.0	.987	47.4
Reward	1935–37	11	13.6	731	1.94	138.5	.970	69.4
Series 2. Comparative tests at northern Alberta stations								
Garnet	1936–38	19	13.4	654	1.83	116.4	.830	52.8
Marquis	1936–38	19	13.6	780	1.56	118.4	.898	68.4
Red Bobs	1936–38	19	12.6	745	1.94	130.0	.966	64.6
Reward	1936–38	19	15.2	906	2.15	156.4	.963	70.0
Series 3. Fertilizer tests on grey soil								
Garnet	1936–38	20	13.1	622	1.84	109.4	.961	57.1
Reward	1936–38	20	15.0	853	2.01	173.9	.955	82.7
Series 4. Miscellaneous tests with Reward wheat								
Reward (Breton)	1936–37	27	14.6	779	1.51	132.2	.892	78.1
Reward (Beaverlodge)	1936	36	13.0	714	1.35	116.8	.973	84.3
Reward (Beaverlodge)	1937	36	15.6	986	0.98	86.4	.831	73.2
Reward (Beaverlodge)	1938	36	15.1	977	0.59	77.1	.641	84.3

All correlation coefficients significant beyond the 1% point.

these results, a difference of 6.3 cc. in the regression coefficients for two varieties is significant. Thus Reward yielded a coefficient that is significantly higher than those for the other three varieties. Even this does not give a complete picture of varietal differences, however, since Garnet does not produce as large a loaf as Red Bobs and Marquis of comparable protein content. This difference can be noted by comparing the mean protein and loaf

volume values for each variety and each year. It is more clearly seen, however, in Fig. 1. Red Bobs and Marquis gave similar regression coefficients

TABLE II

ANALYSIS OF VARIANCE, REGRESSION COEFFICIENTS FOR ALL SERIES CONTAINING THE VARIETIES GARNET, MARQUIS, RED BOBS†, AND REWARD

Variance due to	D.f.	Mean square	*F*
Series	5	280.09	9.4**
Variety	3	536.11	18.0**
Error	15	29.80	
Total	23		

† *One series contained Early Triumph in place of Red Bobs.*

** *Significant beyond the 1% point.*

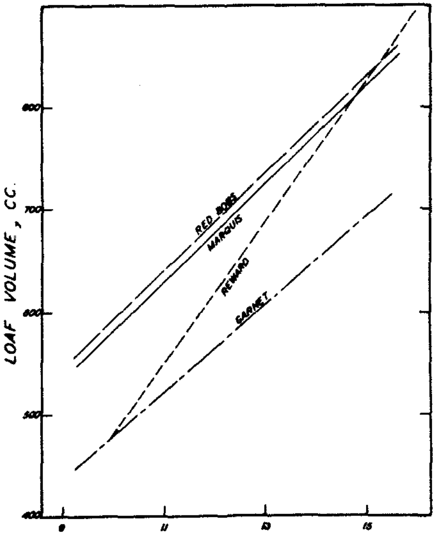

FIG. 1. *Relation between loaf volume and protein content, Series 1, 1935–37.*

and also yield bread of equal volume at any given protein level. Garnet gives about the same regression coefficient, but the regression line falls from 100 to 140 cc. below those for Red Bobs and Marquis. Reward, on the other hand, produces loaves of the same volume as Marquis and Red Bobs at 15% protein, but is little better than Garnet at 10% protein.

Most of the other results given in Table I merely confirm these conclusions. Since the material in Series 2 to 4 was grown in plots from pure seed, there is no question of contamination. In general, the correlation coefficients are somewhat higher than when large numbers of farmers' samples were studied. Particular attention is directed to the consistently high regression coefficients obtained with all series of Reward samples.

Studies with Standard and Rust Resistant Varieties, Series 5

The most comprehensive study was carried out with the co-operatively produced and tested samples of standard and rust resistant wheat varieties. This material came from points widely distributed over the three Prairie Provinces, and the loaf volume results represent the average of four replicate bakings from each of three laboratories. The results of the correlation analysis are presented in Table III.

The results with the standard varieties are in excellent agreement with those discussed in the preceding section. Despite the much greater range in protein content than in the other series, the correlation is linear, and the coefficients are very high. The regression coefficients are of the same general order as before, with no significant difference in the values for Marquis, Early Triumph (essentially Red Bobs), and Garnet, but with Reward much higher. Results for Ceres, a high quality variety, are similar to those for Marquis. The results obtained with Huron are of interest, because this variety is still grown to some extent in Alberta. It is generally considered to be a poor quality wheat, but it has frequently been found that low protein samples made relatively good bread, while high protein samples invariably were poor in quality. This variety, which gave a low regression coefficient, is apparently directly opposite to Reward in its behaviour.

There are now four high quality, licensed rust resistant varieties in Canada, and each of these has been thoroughly tested before being distributed. Three of these varieties gave regression coefficients not significantly different from those for Marquis and Early Triumph. The coefficient for Thatcher is significantly higher than that for Marquis, but the whole regression line lies above the line for Marquis. The steeper slope, therefore, does not indicate poorer quality at any protein level. Two of the rust resistant varieties, Renown and Regent, have Reward as one of the parents, but the steep regression line of Reward is not obtained with either of them. Two other Reward hybrids, however, do show evidence of this tendency, and it is of practical importance that one of these, at least, has been found deficient in quality characteristics. The third unnamed hybrid listed in Table III was discarded from the tests some years ago because it was deficient in loaf volume. That this was justified is shown by the mean protein and loaf volume results.

While it was not as poor in quality as Garnet, the regression line was essentially parallel to that of Marquis but about 70 cc. lower.

The significance of the differences in the regression coefficients for the 13 varieties listed in Table III is shown by the results of an analysis of covariance given in Table IV. These results show conclusively that there are consistent significant differences in the varieties over a period of years.

TABLE III

RESULTS ON SAMPLES GROWN AND TESTED CO-OPERATIVELY, 1933–38

Variety	Years tested	No. of samples	Mean protein ($\bar{p}$), %	Mean loaf volume ($\bar{v}$), cc.	Standard deviation of p, %	Standard deviation of v, cc.	r_{vp}	b_{vp}, cc.
Standard non-rust-resistant varieties								
Garnet	1934–38	11	15.1	797	2.28	152.8	.977	65.4
Marquis	1933–38	13	15.1	893	2.49	151.7	.951	58.0
Early Triumph	1934–37	8	14.6	900	2.43	162.8	.978	65.5
Reward	1933–38	13	16.5	909	2.15	176.9	.983	81.0
Ceres	1933–38	14	15.3	930	2.15	151.7	.950	66.9
Huron	1933–36	9	14.7	822	3.11	125.0	.935	47.8
Named, licensed rust resistant varieties								
Thatcher	1933–38	17	15.6	970	2.31	167.2	.978	71.0
Renown	1933–38	17	15.4	888	2.06	134.6	.970	63.5
Apex	1933–38	17	15.2	887	2.28	138.6	.966	58.8
Regent	1933–38	17	15.5	894	2.27	140.9	.936	58.2
Unnamed, non-licensed rust resistant varieties								
H-44-24 × Reward	1934–38	16	15.6	992	2.25	165.7	.979	72.2
H-44-24 × Reward	1934–38	16	16.3	984	1.87	142.5	.976	74.5
Hope × Reward	1933–35	8	14.2	777	2.15	136.1	.969	61.1
All above varieties	1933–38	176	15.5	926	2.25	160.5	.935	67.0

All correlation coefficients significant beyond the 1% point.

TABLE IV

TEST OF HOMOGENEITY OF VARIETAL REGRESSION COEFFICIENTS BY ANALYSIS OF RESIDUAL VARIANCE, SERIES 5

Variance due to	D.f.	Variance	F
Differences among varietal regression coefficients	12	27,349.4	18.04**
Deviations from individual varietal regressions	150	1,515.9	

** *Significant beyond the 1% point.*

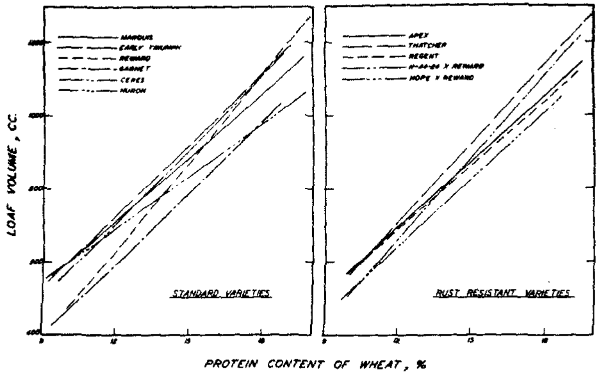

FIG. 2. *Relation between loaf volume and protein content, Series 5.*

The general relations discussed above are illustrated graphically in Fig. 2.

The values for all 13 varieties listed in Table III were included in a single correlation analysis. The results are given at the end of the table. The *high degree of correlation was obtained only because there was such a wide*

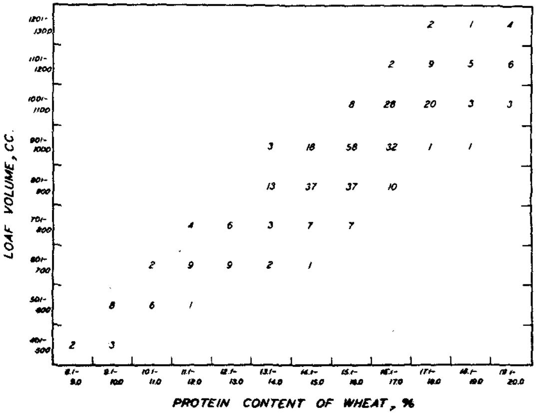

FIG. 3. *Scatter diagram for all results with Series 5.*

range in protein content. The scatter diagram (Fig. 3) made up from the results of all varieties included in these tests (this number varied from 22 to 36 in different years) shows that a range of 400 cc. was not infrequently obtained with different samples varying not more than 1% in protein. The range within individual varieties was rarely more than 150 cc.

Tests for Non-linearity of Regression

Analyses of variance have been carried out with the data for the individual varieties of Series 1, 1936 and 1937, and with the combined data for all varieties in Series 5 (Fig. 3), to test the linearity of the regressions. Only with the latter was any evidence of non-linearity found. The results of this analysis are given in Table V. The F value is significant beyond the 5% point, but

TABLE V

TEST OF LINEARITY OF REGRESSION, SERIES 5 (ALL VARIETIES)

	D.f.	Mean square	F
Deviations, means of arrays from regression line	10	1.070	2.20*
Within arrays	359	0.4872	

* *Significant to the 5% point.*

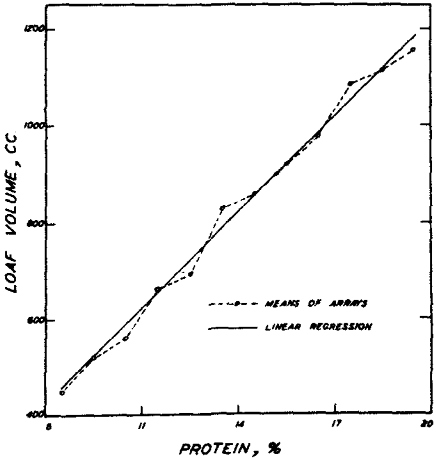

FIG. 4. *Relation between loaf volume and protein content, means of arrays, Series 5, all varieties and years; N = 371.*

when the results for the 13 samples which were above 19% in protein are omitted from the analysis, the *F* value becomes non-significant. The practical unimportance of the significant *F* value is shown by Fig. 4, in which the mean loaf volume for each array (Fig. 3) is plotted against the protein content.

It seems justifiable to conclude, therefore, that for all practical purposes the relation between protein content and loaf volume obtained with the malt-phosphate-bromate formula is linear.

Discussion

The results of this study show that the relation between protein content of wheat and loaf volume of bread is just as much a varietal characteristic as are yield and protein content. The consistently high regression coefficient obtained with Reward wheat serves to explain fully the results that originally led to this investigation. High protein Reward wheat (15% and above) makes bread of large volume, but low protein Reward is not much, if any, better than Garnet. This does not mean that Reward wheat grown under comparable conditions will not produce bread of better quality than Garnet, because under such conditions Reward will usually be about 2% higher than Garnet in protein.

From a purely local point of view, the practical importance of the present results lies in the fact that Reward has been, and is being, extensively used in breeding programs designed to give a high quality variety suitable for the northern areas in which low protein wheat is usually produced. Obviously the decidedly poor quality of low protein Reward wheat is to be regarded as an undesirable characteristic, and any new variety possessing this fault is certain to be unsatisfactory when grown on the poorer soils, no matter how good it appears to be under prairie conditions.

In contrast to Reward, the variety Red Bobs, which is generally regarded as a fairly low protein wheat, has given much better results under northern conditions than would have been expected. This is accounted for by the lower regression coefficient, and the fact that a sample of this variety containing 8% protein will make as good bread as a sample of Reward containing 10%. Such a variety as Garnet, however, is unsatisfactory, not because of a high regression coefficient, but because it requires more protein at all levels to make a loaf of specified volume. For use in the north the plant breeder must aim to produce an early wheat that is free from the undesirable characteristics of both Garnet and Reward.

The present results also indicate that, for the type of wheat produced in Western Canada, the malt-phosphate-bromate formula is very satisfactory for use in testing the baking quality of wheats in so far as the inherent strength characteristics are concerned. The fact that the large seasonal variation in correlation and regression coefficients, noted by several workers, has not been at all apparent in most of the present results, shows that this formula is much more satisfactory with wheats varying widely in protein content than are many of the formulas used by others. It seems likely that many of

the seasonal differences in regression coefficients reported by Waldron and Mangels (11) would have disappeared had such a baking formula been used by them.

Acknowledgments

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VEGETATIVE PROPAGATION OF CONIFERS

V. THE EFFECT OF INDOLYLACETIC ACID AND NUTRIENT SOLUTIONS ON THE ROOTING OF NORWAY SPRUCE CUTTINGS[1]

By N. H. Grace[2] and M. W. Thistle[3]

Abstract

Norway spruce cuttings collected in November 1938 were treated, in three experiments, with a series of solutions containing from 1/729 to 400 p.p.m. of indolylacetic acid. The effects, in all concentrations, were reduced rooting and increased mortality. The addition of nutrient salts to the treating solution further reduced rooting and increased mortality of the cuttings. These negative results of solution treatments contrast with beneficial effects already reported for application of indolylacetic acid in talc dust.

A previous communication in this series described the results of an experiment in which dormant Norway spruce cuttings were treated with indolylacetic acid in a talc carrier (6). This paper deals with experiments in which cuttings, from the same collection, were treated with a range of concentrations of indolylacetic acid in aqueous and in nutrient salt solutions. Preliminary experiments with some 5,000 solution-treated summer and winter cuttings of this species of spruce, by J. L. Farrar and the senior author, suggested that growth stimulating chemicals applied by this method had a damaging effect, at least above a concentration of 5 p.p.m. While lower concentrations did not show as much damage, definite stimulation was not noted. In consequence, the three experiments, to be described, made use of solutions ranging in concentration from 1/729 to 400 p.p.m. (parts per million).

Experimental

The material used in the three experiments was collected in mid-November 1938, the bases of the branches being placed in peat and kept outside under snow (4). Branches were from the upper part of the tree, with one exception to be mentioned subsequently. Cuttings were prepared with a heel of old wood, and ranged in length from 2 to 5 in. Each group of cuttings was representative of the different length classes. Cuttings were treated immediately after preparation. The first experiment was started on January 13, and the second and third experiments on April 7, 1939. All three experiments were carried out in the greenhouse.

Indolylacetic acid solutions were prepared by dissolving 0·1000 gm. in 1 ml. of alcohol and diluting with distilled water to give the highest concentration required. Further dilution permitted ready preparation of the lower

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concentrations. In the first experiment there were two such solutions, one in water and one in a standard nutrient salt solution (5). Freshly prepared solutions were used for all treatments. The cuttings, four replicates of 10 in the first and second experiments and two replicates of 11 in the third, were immersed to a depth of about 3 cm. for 24 hr. in 100 cc. of solution in 400-cc. beakers.

In the first experiment there were 13 main treatments—an untreated control (planted without immersion in water or nutrient solution), a water (or nutrient) control, and a series of 11 concentrations of indolylacetic acid. These descended in geometric progression from 81 to 1/729 p.p.m., each successive concentration being reduced to one-third that of the member of the series immediately preceding. There were eight replicates of each of the foregoing 13 treatments, and the entire experiment required 1040 cuttings. *Four of the replicates were treated with indolylacetic acid in water, the remaining* four in solutions of the chemical in a standard nutrient solution (5). The restricted random arrangement of planting provided, in effect, four double replicates of the 12 remaining treatments, half with and half without nutrients in the treating solution. This arrangement gave two levels of precision, the first appropriate to comparisons between treatments with water and with nutrient salt solution, and the interaction of these with indolylacetic acid, and the second appropriate to comparisons between the 13 main treatments.

The second experiment comprised a series of six treatments—an untreated control, a water treated control, and treatments with 100, 200, 300, and 400 p.p.m. indolylacetic acid in water. This experiment required 240 cuttings.

The third experiment made use of cuttings from the upper and lower regions of the tree and involved three treatment groups—an untreated control, a water treated control, and a group treated with 100 p.p.m. indolylacetic acid in water. There were two replicates of 11 cuttings from each region and 132 cuttings in all. Lower cuttings were from the same tree for which results have been reported on dust treatment (4). However, upper cuttings were from a number of trees, although from the same general collection of material.

Each of the three experiments was planted in the form of simple randomized blocks.

The cuttings were planted in brown sand in propagation frames equipped with bottom heat cables. Sand temperature was maintained at approximately 72° F., while that of the room ranged around 65–70° F. for the first 12 weeks of the first experiment. Room temperature frequently rose to 80 or even 95° F. during the subsequent 12 weeks, the period in which the second and third experiments were in the bed, and prior to the final examination of the first experiment.

The cuttings were examined 12 weeks after planting and the numbers of cuttings rooted, callused, and dead were determined. Record also was made of the numbers and lengths of roots. Cuttings of the first experiment, which were either living or callused but not rooted, were replanted and observed finally at the end of a second period of 12 weeks.

Data on the number of cuttings rooted and the number dead, for Experiments 1 and 3, were subjected to analyses of variance. Only the data on the number dead were analysed for Experiment 2, as the treatments with indolylacetic acid failed to effect any rooting. For this reason, it was also impossible to make any useful statistical treatment of the data on the number and lengths of roots per rooted cutting.

Results

Experiment 1

Data for the analysis of variance and counts of the cuttings rooted and dead are given in Tables I and II. It will be observed that there are significant treatment effects with respect to both counts at both times of observation. In general, significant reduction in rooting is to be noted in practically all cases, owing to the use of indolylacetic acid. Further, all indolylacetic acid dosages produce a significant increase in mortality after both time periods. Counts of the number of cuttings rooted fail to show any interaction between nutrient and indolylacetic acid; in consequence, the results of indolylacetic acid treatment need not be given separately for water and nutrient solution treatments.

TABLE I

EXPERIMENT 1—ANALYSIS OF VARIANCE OF RESPONSE OF NORWAY SPRUCE TO NUTRIENT SALT AND PHYTOHORMONE SOLUTIONS

Source of variance	Degrees of freedom	Mean square			
		After 12 weeks		After 24 weeks	
		Number of cuttings rooted	Number of cuttings dead	Number of cuttings rooted	Number of cuttings dead
Replicates	3	.3129*	1.711***	1.460**	.5102**
Phytohormone treatments	12	.3684**	.678***	1.177**	.2262**
Error (a)	36	.0992	.158	.320	.0801
Water − nutrient differences	1	.6048	.834	9.201***	2.9156***
Interaction (W − N) × phytohormone treatments	11	.0410	.869**	.345	.0368
Error (b)	40	.2235	.306	.586	.1153

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*
All data transformed, $\sqrt{x + \frac{1}{2}}$ *basis (1).*

The effect of nutrient is one of damage throughout the experiment. Nutrient damage is not apparent as such at the 12-week period, except as it accentuates the increase in mortality caused by indolylacetic acid. After 24 weeks, however, injury from nutrient is strongly marked, only 13% rooting after nutrient, as against 23% after treatment with the corresponding aqueous solutions; 80% mortality with nutrient as contrasted with 61% mortality with water is also noted.

TABLE II

EXPERIMENT 1—RESPONSE OF NORWAY SPRUCE TO PHYTOHORMONE SOLUTIONS

Each figure is a mean value for 80 cuttings

Indolylacetic acid *concentrations*	Transformed data				Data in percentages			
	12 weeks		24 weeks		12 weeks		24 weeks	
	Cuttings rooted	Cuttings dead	Cuttings rooted	Cuttings dead	Cuttings rooted	Cuttings dead	Cuttings rooted	Cuttings dead
81 p.p.m.	.96*	2.34*	1.36*	2.88*	5	52	19	70
27 p.p.m.	.95*	1.87*	.85*	2.76*	5	36	12	74
9 p.p.m.	1.20*	1.82*	1.65	2.68*	11	32	24	68
3 p.p.m.	1.07*	1.87*	1.11*	2.81*	8	34	18	75
1 p.p.m.	.84*	1.92*	.85*	2.81*	2	34	9	75
1/3 p.p.m.	1.33	1.74*	1.66	2.63*	14	26	24	65
1/9 p.p.m.	1.12*	1.87*	1.20*	2.72*	9	34	16	70
1/27 p.p.m.	1.09*	1.64*	1.09*	2.85*	8	28	12	78
1/81 p.p.m.	1.01*	1.75*	1.19*	2.76*	6	30	20	72
1/243 p.p.m.	1.12*	1.65*	1.17*	2.64*	9	25	15	66
1/729 p.p.m.	1.04*	1.80*	1.04*	2.67*	8	30	15	68
H_2O only	1.47	1.42	1.81	2.41	20	19	34	54
Untreated	1.60	1.07	2.11	2.29	24	8	41	49
Necessary difference	.32	.40	.57	.29				

* *Differs significantly from untreated control.*

Experiment 2

The results of an analysis of variance of the data on the number of cuttings dead are presented in Table III, and it is apparent that treatment has had a highly significant effect. In Table IV are given data showing that the effect was injurious in all cases.

The untreated group had significantly fewer dead cuttings than the water control or any of the indolylacetic acid treated groups. The 100 p.p.m. treatment group had more dead cuttings than the water treated, but fewer than the groups receiving the higher concentrations, which did not differ

TABLE III

EXPERIMENT 2—ANALYSIS OF VARIANCE OF THE PROPORTION OF NORWAY SPRUCE CUTTINGS DEAD ON TREATMENT WITH PHYTOHORMONE SOLUTIONS

Source of variance	Degrees of freedom	Mean square
Blocks	3	51.28
Treatments	5	2488.52***
Error	15	69.26

Data transformed to angles (inverse sine) for analysis of variance (1).

****Exceeds mean square error, 0.1% level of significance.*

TABLE IV

EXPERIMENT 2—THE EFFECT OF PHYTOHORMONE SOLUTIONS ON THE RESPONSES OF NORWAY SPRUCE CUTTINGS

Each figure is a mean value for 40 cuttings

	Untreated	Indolylacetic acid in water, p.p.m.					Necessary difference, 5% level
		0	100	200	300	400	
No. of cuttings dead—							
Transformed* data	31.0	46.5	72.1	85.4	90.0	90.0	12.5
Per cent	28	53	88	98	100	100	
No. of cuttings rooted and callused, %	60	43	3	2	0	0	
No. of cuttings rooted, %	5	25	0	0	0	0	

* *Data transformed to angles for analysis of variance (1).*

significantly among themselves. None of the groups treated with indolylacetic acid contained any rooted cuttings, whereas appreciable rooting and callus formation occurred in the two groups without chemical treatment.

Experiment 3

Table V gives the results of analyses of variance of the number of cuttings rooted and dead, which demonstrate the existence of significant positional and treatment effects, and the means in Table VI show the nature of these effects. Cuttings from the lower part of the tree gave significantly better rooting and fewer dead cuttings than those taken from the upper region. While the untreated and water treated groups failed to differ significantly with respect to either the number of cuttings rooted or dead, both groups differed from the

TABLE V

EXPERIMENT 3—ANALYSIS OF VARIANCE OF RESPONSE OF NORWAY SPRUCE CUTTINGS FROM THE UPPER AND LOWER PART OF THE TREE TO TREATMENT WITH PHYTOHORMONE SOLUTION

Source of variance	Degrees of freedom	Mean square	
		Number of cuttings rooted†	Number of cuttings dead†
Blocks	1	654.16*	68.64
Treatments	2	723.55*	2332.14***
Position of cutting on tree	1	3240.65**	579.84*
Interaction treatment × position	2	195.59	60.31
Error	5	78.86	50.02

† *Data transformed to angles for analysis of variance (1).*
* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

TABLE VI

EXPERIMENT 3—RESPONSES OF NORWAY SPRUCE CUTTINGS FROM THE UPPER AND LOWER PART OF THE TREE TO TREATMENT WITH PHYTOHORMONE SOLUTION

Each figure is a mean value for 22 cuttings

	Position of cutting on tree		Necessary difference, 5% level	Untreated	Water only	Indolyl-acetic acid, 100 p.p.m.	Necessary difference, 5% level
	Upper	Lower					
Number of cuttings rooted							
Transformed* data	7.1	40.0	13.2	26.9	35.0	8.8	16.1
Per cent	4	42		27	39	5	
Number of cuttings dead							
Transformed* data	44.7	30.6	10.5	19.9	27.8	65.2	12.9
Per cent	48	32		16	23	82	

* *Data transformed to angles for analysis of variance (1).*

one receiving the 100 p.p.m. indolylacetic acid treatment. This concentration of indolylacetic acid greatly reduced rooting and increased the number of cuttings that died.

Discussion

The results of the three experiments have indicated that indolylacetic acid solutions from 1/729 to 400 p.p.m. reduced the rooting and increased mortality of Norway spruce cuttings. This finding is in agreement with that of Deuber and Farrar (2, 3). However, Thimann and Delisle have reported increased rooting of Norway spruce cuttings on solution treatment (7). It is possible that some unrecognized factor is responsible for this disagreement, since the rather marked differences between replicates noted in the propagation of conifer cuttings suggest that they are highly sensitive to apparently minor variations.

Treatment in solutions of nutrient salts for 24 hr. reduced rooting and increased mortality. Apparently this method of applying nutrient salts is too drastic, for it has been shown that small additions to the sand in which the cuttings are propagated increased rooting and decreased mortality (5).

Cuttings from the lower part of the tree gave better rooting and lower mortality than those taken from the upper region, a result in agreement with earlier findings (4). However, treatment with 100 p.p.m. indolylacetic acid solution was equally injurious to cuttings from both regions of the tree.

In Experiment 2 water treatment increased mortality over that obtained with untreated cuttings; however, water treatment also increased rooting appreciably. The explanation is found in the large number of cuttings that were merely callused. The number of cuttings that were rooted and callused was reduced by water treatment. In Experiments 1 and 3 water treatment was without significant effect.

The further increase in injury after indolylacetic acid solution treatment may be due to changes in surface tension, osmotic relations at the cell membranes, or other physico-chemical factors. In Table II it was shown that significantly more cuttings were rooted at 24 weeks following a 1/3 p.p.m. treatment than following one in which 1/729 p.p.m. of indolylacetic acid was present. The greater damage at the lower concentration suggests that absorption of toxic amounts of the chemical is not the sole cause of injury.

While solution treatment of Norway spruce cuttings with indolylacetic acid has been damaging, it must be recalled that 1000 p.p.m. in talc dust had a beneficial effect (6).

Acknowledgment

The writers wish to acknowledge their indebtedness to Dr. J. W. Hopkins for his assistance in planning the statistical arrangement of the experiment.

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QUALITATIVE STUDIES OF SOIL MICRO-ORGANISMS

IV. THE RHIZOSPHERE IN RELATION TO THE NUTRITIVE REQUIREMENTS OF SOIL BACTERIA[1]

BY P. M. WEST[2] AND A. G. LOCHHEAD[3]

Abstract

Bacteria of the rhizospheres of flax and tobacco were found to possess more complex nutritive requirements than those in the corresponding control soils. The roots of even young seedlings favour the development of those types that are dependent upon a supply of thiamin, biotin, and amino nitrogen for their growth, thus suggesting that the roots may excrete significant amounts of these stimulative substances. The "rhizosphere effect" was more pronounced with susceptible than with resistant varieties of either flax or tobacco. A greater difference was found to exist between the rhizosphere and the control soil where the latter is poor than where it is richly supplied with organic matter, since liberation of growth substances by plant decomposition permits a limited development of the more typically rhizosphere forms, apart from the zone of influence of the growing plant.

Introduction

On the basis of cell morphology and taxonomic relationship, Lochhead (2) found qualitative differences between bacteria in the rhizosphere and in soil distant from plant roots. It was observed also that the growing plant appeared to be a greater factor in determining the relative predominance of different morphological types of micro-organisms in soil of definite type than did various fertilizer treatments, which, as had been shown by Taylor and Lochhead (3), exerted relatively little effect on the incidence of the various groups of bacteria. These results suggested that the plant, through the agency of root excretions, might play an important role in the nutrition of rhizosphere forms, and that in addition to differences in the relative occurrence of various morphological types in the rhizosphere compared to normal soil, there might also be significant differences in the nutritive requirements of the organisms in the two groups. This possibility was investigated, since the existence of any specific nutritive differences would not only give some indication concerning the nature of the heretofore hypothetical "plant excretions", but also explain, at least in part, the mechanism by which the plant induces the development of the characteristic rhizosphere flora.

Experimental

From the rhizospheres of Bison and Novelty flax, resistant and susceptible respectively to wilt, 100 representative bacteria were isolated according to quanti-qualitative methods previously described (2, 3). The plants were grown under greenhouse conditions in pots containing uniform soil. One hundred control organisms were isolated from the pot soil after the roots, and

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the soil adherent to them, had been removed. These organisms were inoculated into four media of increasing complexity.

(1) Base medium consisting of glucose 1.0 gm., K_2HPO_4 1.0 gm., KNO_3 0.5 gm., $MgSO_4$ 0.2 gm., $CaCl_2$ 0.1 gm., NaCl 0.1 gm., $FeCl_3$ 0.01 gm., distilled water, 1 litre. Reaction, pH 6.8.

(2) Base medium enriched with amino nitrogen: asparagine 0.5 gm., aspartic acid 0.1 gm., cysteine 0.1 gm., glycine 0.1 gm., alanine 0.1 gm. per litre.

(3) Base medium enriched with amino nitrogen, (as above) together with a combination of bacterial growth factors: thiamin (vitamin B_1) 0.1γ, biotin 0.0005γ, β-alanine 0.1γ, nicotinic acid 0.1γ, and i-inositol 0.1 mg. per ml.

(4) Base medium enriched with yeast extract 1.0 gm. per litre.

All amino acids and growth factors were used in pure form with the exception of biotin, available as a concentrate prepared by the procedure of Kögl and Tonnis (1).

The complexity of the nutritive requirements of the bacteria from rhizosphere and control soils was determined by their growth responses in the above media after five days' incubation at 28° C. The simplest requirements were shown by those organisms producing heavy growth in the base medium, and growing no more abundantly in the media to which supplements had been added. Others, while capable of development at a sub-maximal rate in the base medium, grew more rapidly on one or more of the other substrates. The most fastidious organisms were quite unable to grow in the base medium, and in these cases the presence of amino nitrogen or growth factors was absolutely essential.

Requirements of Bacteria from Flax Rhizosphere

As shown in Table I and Fig. 1, in comparison to the controls, organisms from the rhizosphere of Bison (resistant) showed an 83% proportionate increase in numbers for which amino nitrogen was either stimulative or essential, while the organisms from Novelty (susceptible) showed a 325% increase. The numbers of bacteria influenced by the combined growth factors were increased 71% in the rhizosphere of Bison, and 143% in the rhizosphere of Novelty, beyond their incidence in the control soil. These findings indicate that in addition to the morphological differences observed (2) between the predominant types in rhizosphere and control soils, there also occurs a marked difference in the nutritive requirements of the organisms in the two groups. The greater proportion of the bacteria in the flax rhizosphere appear to depend on a supply of certain amino acids or growth substances for their maximum development, while the majority of the organisms in the check soils are independent of these factors for their growth. Furthermore, it is apparent that the relative incidence of various nutritional forms differs more widely from the control soil in the rhizosphere of the susceptible flax variety than in the rhizosphere of the resistant.

TABLE I

RELATIVE INCIDENCE OF BACTERIA OF VARIOUS NUTRITIVE TYPES IN RHIZOSPHERE OF FLAX AND TOBACCO AND IN CONTROL SOILS

—	Flax			Tobacco		
	Control	Rhizosphere		Control	Rhizosphere	
		Res.	Susc.		Res.	Susc.
	%	%	%	%	%	%
Growth in base medium	2.1	49.4	39.7	43.0	36.0	50.0
Amino acids essential for growth	14.7	16.9	44.1	11.8	19.5	29.0
Amino acids stimulative	0.0	10.4	19.1	6.4	13.4	22.0
Either essential or stimulative	*14.7*	*27.3*	*63.2*	*18.2*	*32.9*	*51.0*
Growth factors essential	25.6	26.4	48.2	24.7	34.0	41.8
Growth factors stimulative	*4.0*	*24.3*	*23.8*	*12.7*	*14.4*	*19.7*
Either essential or stimulative	29.6	50.7	72.0	37.4	48.4	61.5
Stimulated by yeast extract only	*67.8*	*19.5*	*14.7*	*26.7*	*26.8*	*6.9*
No stimulation in any medium	2.1	30.0	13.2	34.2	22.6	25.5
Amino acids and growth factors *without effect*	69.9	49.5	28.0	61.0	49.4	32.4

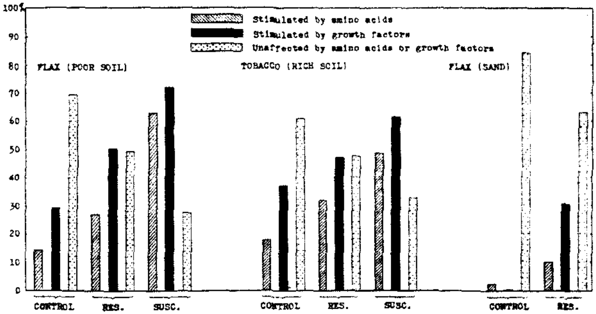

FIG. 1. *Relative incidence of rhizosphere bacteria in relation to certain nutritive requirements.*

Requirements of Bacteria from Tobacco Rhizosphere

The above experiments were repeated with bacteria from the rhizosphere of another crop, grown under field conditions. Two varieties of tobacco, one resistant and the other susceptible to black root rot, were selected for this study. As previously described, 100 isolates from the rhizospheres of resistant and susceptible tobacco varieties, together with 100 controls, were inoculated into each of the four differential media. The results of this com-

parison are also shown in Table I and Fig. 1. The rhizosphere of resistant plants showed *an increase of 80% in the relative incidence of bacteria stimulated* by amino acid nitrogen, while the susceptible plant rhizosphere showed an increase of 180% compared to the control soil. The number of organisms responding to the medium containing growth factors was increased 29% in the rhizosphere of resistant tobacco, and 64% in the rhizosphere of susceptible tobacco, beyond their incidence in control soil. These results are essentially in agreement with those obtained with flax, which suggests that the same fundamental factors are operative in both cases. As the tobacco soil was relatively rich, however, considerably more thiamin and biotin were already present from decomposing plant residues, thus minimizing somewhat the rhizosphere effect exerted by the plant. The poorer soil in which flax had grown therefore showed the same result in a more accentuated manner.

Relation of Control Soil to Rhizosphere Bacteria

In order to avoid entirely the interfering effect of growth substances already contained in the soil, sand was substituted in another experiment, so that the only source of growth stimulants could be the growing plant. The sand was sterilized in pots, then planted with Bison flax seedlings and inoculated with pure cultures of soil organisms, some requiring accessory growth substances, others requiring amino acids, and others growing well on the simple base medium. The initial total count, which includes approximately equal numbers of each nutritive type, was 12,800,000 per gram of sand. After three weeks in the greenhouse, representative organisms were isolated from the flax rhizosphere, together with controls from corresponding pots in which no plants had grown. The control sand contained 33,000,000 organisms per gram, but only 2% of 100 representative organisms required amino acids for their growth, while no organisms for which growth factors were essential had survived at all. On the other hand, the rhizosphere contained 1,035,000,000 organisms per gram, and, as shown in Fig. 1, approximately *one-third of the total numbers was represented by bacteria for which a* supply of growth factors is essential.

Thus it appears that unless the more fastidious organisms of the rhizosphere have access to certain organic substances, their development, especially among other forms which are limited by an external supply of these stimulants, is almost impossible. As has been demonstrated above, the numbers of organisms with specific growth factor requirements that occur normally in soils are therefore related in a general way to the organic matter content of the soil in question. The rich tobacco soil supported relatively high numbers, the poorer flax soil fewer numbers, and the infertile sand supported none of the bacteria having more complex nutritive requirements (Fig. 1). Even in sand, however, the predominant types of bacteria immediately adjacent to the plant roots are the more complex forms.

Essential Amino Acids

Organisms for which amino nitrogen was essential were selected for further study, in order to determine which of the ingredients of the amino acid enriched medium was specifically responsible for the observed effects. When each amino acid was tested singly no growth occurred in any case, with the possible exception of cysteine, which produced a barely detectable response. Apparently then, a combination of amino acids was necessary to yield the marked stimulative effects previously observed. On the assumption that cysteine was an essential part of such a combination, this acid was tested with each of the other ingredients of the medium. Results for two typical organisms of this group, shown in Table II, indicate that either alanine or asparagine is effective in combination with cysteine, while aspartic acid and glycine are relatively inert. By varying the concentration of cysteine in the presence of a constant amount of alanine, the optimum effect of the former was observed over the range 0.005 to 0.10 gm. per litre. Higher concentrations of cysteine than 0.01% were distinctly inhibitory. At no concentration could thioglycollic acid replace the amino acid.

TABLE II

COMBINED EFFECT OF CERTAIN AMINO ACIDS ON SELECTED ORGANISMS FROM THE RHIZOSPHERE

Additions to base medium in gm. per l.	Organism	
	XVIII-33	XVIII-21
Asparagine, 0.5	–	–
Aspartic acid, 0.1	–	–
Cysteine, 0.1	±	±
Glycine, 0.1	–	–
Alanine, 0.1	–	–
Ammonium chloride, 0.1	–	–
Cysteine, 0.1 + aspartic acid, 0.1	±	±
Cysteine + asparagine, 0.5	++++	++++
Cysteine + glycine, 0.1	++	+
Cysteine + alanine, 0.1	++++	++++
Cysteine + ammonium chloride, 0.1	+	+
Combination of all above amino acids	++++	++++

An examination of 16 pure amino acids was then made to determine whether any other sources of amino nitrogen, in combination with cysteine, were capable of providing the essential conditions for growth of this group of rhizosphere bacteria. The results of this experiment, presented in Table III, indicated that the only other amino acids able to replace alanine or asparagine were proline or hydroxyproline, the latter possessing somewhat less activity than the others. All exerted full stimulative effect as low as 0.05 gm. per litre, becoming inactive at approximately one-tenth that concentration.

The interesting question concerning whether the plant provides the rhizosphere organisms with one of these essential combinations naturally arises.

TABLE III

AMINO ACID REQUIREMENTS OF BACTERIA FROM THE RHIZOSPHERE

Additions to base medium in gm. per l.	Growth after 5 days at 28° C.		
	XVIII-33	XVIII-21	XVIII-32
Cysteine, 0.1	±	±	±
Cysteine + glycine, 0.1	+	+	++
Cysteine + alanine, 0.1	++++	++++	++++
Cysteine + serine, 0.1	±	±	±
Cysteine + valine, 0.1	±	±	±
Cysteine + leucine, 0.1	±	±	±
Cysteine + phenylalanine, 0.1	±	±	±
Cysteine + tyrosine, 0.1	±	±	±
Cysteine + tryptophane, 0.1	±	±	±
Cysteine + proline, 0.1	++++	++++	++++
Cysteine + hydroxyproline, 0.1	++++	+++	+++
Cysteine + aspartic acid, 0.1	±	±	±
Cysteine + glutamic acid, 0.1	±	±	±
Cysteine + histidine, 0.1	±	±	±
Cysteine + arginine, 0.1	±	±	±
Cysteine + lysine, 0.1	±	±	±
Cysteine + asparagine, 0.1	++++	++++	++++

In Table I it will be noted that 44% of the total numbers of organisms in the rhizosphere of Novelty flax found some such effective combination of amino acids or its equivalent, essential to their growth. The significance of these specific nutritive requirements in the light of possible plant excretions must await further study.

Essential Growth Factors

A determination of the active ingredients in the more complex medium, which contained, in addition to amino acids, a mixture of accessory growth factors, was carried out in a manner similar to the above. Each growth factor, when added to the base medium singly, was without effect. Further experiments showed that the activity of the growth factor mixture was dependent on the presence of cysteine, none of the other amino acids being of importance for this group. Using a base medium containing 0.01% cysteine, each growth substance was tested singly on the bacteria for which growth substances had been found either essential or stimulative, with the results presented in Table IV. This table includes the effect of the accessory growth factors on representative organisms from the rhizospheres of both flax and tobacco. In every case it will be noted that the active ingredient in this medium is either thiamin or biotin or both. β-Alanine, nicotinic acid, and inositol do not appear to be of significance in the nutritive needs of these bacteria. Again the possibility of root excretions suggests itself as an explanation for the relative abundance of thiamin- and biotin-requiring organisms in the rhizosphere compared to soil apart from the plant. Since the rhizosphere

TABLE IV

GROWTH FACTOR REQUIREMENTS OF 25 REPRESENTATIVE RHIZOSPHERE BACTERIA

Organism	Base medium + cysteine, 0.1 gm. per l.					
	Control	Thiamin, 0.1γ per ml.	Biotin, 0.0005γ per ml.	β-Alanine, 0.1γ per ml.	Nicotinic acid, 0.1γ per ml.	i-Inositol, 0.1 mg. per ml.
Flax						
XVI-1	+	+++	++++	++	+	+
XVI-25	−	++++	++++	±	−	−
XVI-43	−	++++	+++	+	−	−
XVI-72	−	+	++++	−	−	−
XVI-82	±	+	++++	+	+	±
XVI-91	−	++++	+++	±	±	−
XVII-5	−	++++	−	−	−	−
XIX-1	−	++++	++++	−	−	−
XVIII-2	−	++	++++	−	−	−
XVI-8	±	±	++++	±	±	±
XVI-13	++	+++	++++	++	++	++
XVI-32	++	+++	++++	++	++	++
XVI-36	+	+++	++++	+	+	+
Tobacco						
C62	−	−	++++	−	−	−
C68	−	−	++++	−	−	−
C89	−	++	++++	−	−	−
C 5	−	−	++++	−	−	−
C19	−	+++	++++	−	−	−
R11	−	−	++++	−	−	−
R82	−	−	++++	−	−	−
S66	−	−	++++	−	−	−
S22	−	++++	++++	−	−	−
S77	−	++	++++	−	−	−
B16	−	++++	−	−	−	−
B 9	−	−	++++	−	−	−

effect is exerted by the plant even in the seedling stage, it appears that the liberation of growth substances into the rhizosphere occurs more probably by excretion than by decomposition. More recent research has yielded direct evidence in support of this view (4).

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NITRIFICATION UNDER AND AFTER ALFALFA, BROME, TIMOTHY, AND WESTERN RYE GRASS

III. COMPOSITION OF HAY CROP RESIDUES[1]

By R. Newton[2] and R. S. Young[3]

Abstract

Proximate analyses of roots (to plow depth) and stubble in one-, three-, and five-year-old sods, considered in relation to sequence effects as judged by the nitrogen absorption of the first two wheat crops after each age of sod, indicated the nitrogen content of the hay crop residues to be the dominant influence. Alfalfa was much superior to the grasses, a result apparently of the higher quantity of nitrogen returned to the soil and of the narrower C : N ratio in its residues. Timothy led the grasses, contributing the highest quantity of nitrogen in residues with the lowest percentage of crude fibre and the narrowest ratio of crude fibre to nitrogen-free extract. Brome contributed more residual nitrogen than western rye, but was slightly inferior in sequence effects.

In the first paper of this series (2) experiments at Edmonton, Alberta, extending over a period of eight years were reported, in which the nitrogen absorption of alfalfa (*Medicago sativa* L.), brome (*Bromus inermis* Leyss.), timothy (*Phleum pratense* L.), and western rye grass (*Agropyron tenerum* Vasey), and of succeeding wheat crops, was used as an indication of the rate of soil nitrification. The stubble and roots (to six-inch depth) of these crops were included in the estimations of yield and nitrogen absorption. It will now be appropriate to examine in greater detail the quantity and composition of the hay crop residues, in relation to the rates of nitrification found after breaking one-, three-, and five-year-old sods.

Analytical Data

The roots and stubble were subjected to proximate analyses by standard methods. Though the roots had been carefully washed before drying and grinding for analysis, the apparent ash content of the grass roots was so high as to lead to further examination of some of the original material. Under a low-power microscope it could be seen that fine particles of sand were so firmly embedded in the surface of the roots as to be impracticable of washing out. The analytical results are therefore expressed in Table I on the basis of organic matter as found by combustion. The table is defective with respect to western rye grass, five-year-old sod being unavailable because

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TABLE I

COMPOSITION OF ORGANIC MATTER IN ROOTS AND STUBBLE

Sod	Age of sod, yr.	Crude protein, %		Ether extract, %		Crude fibre, %		N-free extract, %	
		Roots	Stubble	Roots	Stubble	Roots	Stubble	Roots	Stubble
Alfalfa	1	13.0	17.0	3.54	.98	26.3	41.0	57.2	41.0
	3	13.6	11.0	1.09	.75	28.1	46.6	57.2	41.6
	5	15.1	15.1	1.28	.74	29.4	43.2	54.3	40.9
Brome	1	7.2	8.9	.43	1.42	35.8	39.3	56.6	50.3
	3	7.6	4.0	.18	.60	31.2	37.2	61.0	58.2
	5	7.0	4.3	.67	—	32.6	—	59.7	—
Timothy	1	6.4	6.2	.94	1.91	31.9	25.6	60.7	66.3
	3	10.3	5.8	.57	1.18	30.8	28.2	58.3	64.9
	5	7.1	3.3	.65	.70	26.1	28.0	66.2	68.0
Western rye	1	9.2	6.5	1.60	2.19	35.6	41.2	53.6	50.1
	3	11.1	6.4	.49	.63	33.5	38.3	54.9	54.7

killed by "take-all"; also with respect to brome grass stubble from five-year-old sod, some of the analyses being omitted because the abnormally low yield of stubble (see Table II) provided insufficient material in the sod samples taken before plowing.

The alfalfa residues were obviously much richer in protein than the grass residues. The grass stubble also shows a greater tendency to become leaner in the older sods, possibly owing to the inclusion of leached residues from earlier hay cuttings. Western rye roots were higher in protein than those of the other grasses in the two cases in which comparison is possible; in one of these cases its stubble was also richer; but the variability of the results makes caution necessary in their interpretation.

The mixed fatty substances represented by the ether extract occurred in variable but generally small concentration. While it is known that fats decompose slowly in the soil and it has been suggested that infertility may result from their accumulation (3, p. 463–5), it seems safe to assume that any differential effect of these hay crop residues arising from such a cause during the comparatively short period of this experiment would be negligible. Ether extract will therefore be dismissed from further consideration in this paper.

Crude fibre was generally lower in the alfalfa than in the grass roots, while in the stubble the situation was reversed. Among the grass roots there were no striking differences in this respect, though timothy was lowest, especially in the five-year-old sod. In the stubble, timothy had definitely less crude fibre than the others.

In Table II the quantities of organic matter and nitrogen returned to the soil by the residues are compared with the nitrogen absorbed by two succeeding wheat crops. In one-year-old grass sods the stubble exceeded the

TABLE II

CONSTITUENTS RETURNED TO SOIL IN SODS, IN RELATION TO NITROGEN ABSORBED BY TWO SUCCEEDING WHEAT CROPS

Sod	Age of sod, yr.	Organic matter, lb./acre			Nitrogen, lb./acre		
		Roots	Stubble	Total	In residues	In first wheat crop	In first + second wheat crops
lfalfa	1	3847	2134	5981	138	67	120
	3	7304	657	7961	171	104	144
	5	6737	637	7374	178	66	132
	Mean	5963	1143	7105	162(154)*	79(85)*	132(132)*
rome	1	2036	2497	4533	59	54	107
	3	*6564*	*408*	*6972*	*82*	65	104
	5	4488	199	4687	52	52	103
	Mean	4363	1035	5397	64(70)*	57(60)*	105(105)*
imothy	1	2573	2814	5387	54	48	101
	3	5695	811	6506	102	85	134
	5	6538	595	7133	77	58	119
	Mean	4935	1407	6342	78(78)*	64(66)*	118(118)*
estern rye	1	901	2901	3802	43	56	107
	3	3179	1263	4442	69	66	108
	Mean	2040	2082	4122	56	61	108

* *Means of one- and three-year-old sods in parentheses.*

oots in quantity, strikingly so in western rye grass. In three- and five-year- ld grass sods the relation was strongly reversed. Alfalfa roots exceeded he stubble at all three ages. If the entire grass root system had been meas- ired, rather than only the part contained to six-inch depth, the brome and imothy roots also would undoubtedly have exceeded the corresponding stub- les at all ages*. Therefore in considering the significance of these residues, nore weight must be attached to the root analyses than to the stubble analyses.

In general, *the weight of organic matter in the residues reached a maximum* n three-year-old sods, though timothy continued to make some later gain. lfalfa excelled the grasses in weight of organic residues, a superiority that is nuch enhanced in terms of nitrogen, by reason of the higher percentage of rotein in the alfalfa residues. Only alfalfa returned to the soil much more han enough nitrogen for the immediately succeeding wheat crop. Actually lfalfa returned more than enough for two succeeding wheat crops.

The ratios of carbohydrate to protein and of crude fibre to nitrogen-free xtract, given in Table III, must be considered as expressions of factors

* *Professor T. K. Pavlychenko, University of Saskatchewan, Saskatoon, in a personal com- unication states that he has usually found in grass sods about two-thirds of the total root material n the top six inches.*

TABLE III

RATIO CARBOHYDRATE TO PROTEIN AND CRUDE FIBRE TO NITROGEN-FREE EXTRACT IN HAY CROP RESIDUES

Sod	Age of sod, yr.	Ratio carbohydrate* to protein			Ratio crude fibre to nitrogen-free extract		
		Roots	Stubble	Total	Roots	Stubble	Total
Alfalfa	1	6.4	4.8	5.7	.40	1.00	.61
	3	6.3	8.0	6.4	.49	1.12	.53
	5	5.6	5.6	5.6	.54	1.06	.57
Brome	1	12.9	10.0	11.1	.63	.78	.71
	3	12.1	24.0	12.5	.51	.64	.52
	5	13.2	22.0†	13.4	55	—	—
Timothy	1	14.4	14.8	14.6	.53	.39	.45
	3	8.6	16.2	9.2	.53	.43	.52
	5	13.1	29.0	13.7	.39	.41	.40
Western rye	1	9.7	14.1	12.7	.66	.82	.78
	3	8.0	14.5	9.2	.61	.70	.64

* *Carbohydrate taken as sum of crude fibre and nitrogen-free extract.*

† *To make this calculation, the missing value for ether extract in Table I was taken as 0.70.*

modifying the rate and manner of decomposition of the residues. Protein-rich residues should decompose rapidly, with little temporary immobilization of soil nitrates by assimilation in the bodies of micro-organisms engaged in the destruction of carbonaceous material. Crude fibre is a resistant fraction, while nitrogen-free extract contains the readily fermentable, energy-yielding substances. Narrow ratios for both of these factors should therefore indicate favourable conditions for nitrification.

Table III shows that the ratio of carbohydrate to protein is much narrower in alfalfa than in the grasses. Among the grasses, any differences in this respect are obscured by the variations in the results. The roots of brome and timothy seem much alike, while those of western rye, as far as can be judged by the one- and three-year-old sod samples, have a narrower ratio. It also appears that in sods more than one year old the grass roots have a much narrower ratio than the stubble. The grass stubble shows a progressively wider ratio with increasing age, a corollary of the behaviour in regard to protein content noted earlier.

With respect to the ratio of crude fibre to nitrogen-free extract, the only consistent difference between alfalfa and the grasses is the higher value in alfalfa stubble. Generally speaking, this is offset by a narrower ratio in the alfalfa roots. Among the grasses, timothy is generally lowest, especially in stubble and combined residues, in which it has the lowest ratios of all the crops. Western rye, on the other hand, is generally highest.

Discussion

Obviously nitrification in the soil used in these experiments was by no means wholly dependent for its substrate on the residues of the immediately preceding hay crops. The fertile, black Edmonton soil in question is high in organic matter and nitrogen. Analyses recorded in the second paper of this series (2, Table XXV) give the following mean values of 83 determinations of total nitrogen percentage in each of the main soil levels during the course of the investigation: surface, 0.60; subsurface, 0.28; subsoil, 0.10. On the basis of a specific gravity of 1.1 for surface soil, 1.4 for subsurface, and 1.5 for subsoil, there would be nearly 9,000 lb. of nitrogen per acre in the top 6 in. and over 28,000 lb. in the top 40 in. of soil. A seasonal nitrification of 50 to 100 lb. per acre, as measured by absorption into the wheat plants, seems negligible by comparison. But the main bulk of organic matter in the soil consists of an accumulation, over a long period of time, of the more resistant fractions of plant residues, together with the products of microbial growth on these residues. Waksman (4, p. 127) believes humus nitrogen to be held in lignin-protein complexes, decomposing only slowly. Fresh residues may thus have an influence on immediate fertility quite disproportionate to their relative quantity.

In the first paper of this series (1) it was shown that the mean nitrogen absorption per acre of wheat plants following one-, three-, and five-year-old sods for six, four, and two successive years, was: after alfalfa, 63.4 lb.; after timothy, 58.6 lb.; after western rye, 56.3 lb.; after brome, 51.9 lb. The second paper (2) showed that when the sods were plowed up, nitrates accumulated in the alfalfa plots to a greater extent than in the grass plots, and to a lesser extent generally in the brome plots than in the timothy and western rye plots. In the present paper comparisons of after effects of the hay plant residues are restricted to the first two years following each age of sod. On this basis the pre-eminence of alfalfa is definitely confirmed by Table II, and the grasses on the average fall in the order just indicated, though with substantial deviations in individual cases.

The dominant influence of nitrogen content of residues is clearly shown by a comparison of alfalfa with the grasses, though the relative importance of absolute quantity and of ratio to other constituents is not so easily discernible, since alfalfa not only excels in quantity of nitrogen but has also the narrowest ratio of carbohydrate to protein. Among the grasses, timothy residues in three-year-old sod possessed the highest quantity of nitrogen, also a comparatively low C : N ratio, and were followed by the highest wheat-nitrogen absorption.

Pound for pound, the nitrogen of western rye residues seemed more completely assimilated by succeeding wheat crops than that of any other of these hay crops, not excepting alfalfa. This may signify merely that because western rye residual nitrogen was smallest in quantity, succeeding crops drew more heavily on other organic nitrogen reserves in the soil. The fact that

alfalfa, with the highest quantity of residual nitrogen, had the lowest percentage of this absorbed by the first wheat crop, and that timothy occupies a corresponding position among the grasses, lends colour to this view.

To compare the sequence effects of all three grasses in Table II, one must look only at the wheat-nitrogen absorption following one- and three-year-old sods. Brome was on the average slightly inferior to western rye and definitely inferior to timothy. This order does not quite correspond with that of the relative quantities of nitrogen supplied by their residues, which was: timothy, brome, western rye. Brome had a wider C : N ratio in its roots than western rye, though a slightly narrower ratio of crude fibre to nitrogen-free extract in both roots and stubble. One case in which the nitrogen absorption of the first wheat crop is distinctly less than would be expected from the return by the grass residues is that after three-year-old brome. In this case the brome residues had a decidedly wider C : N ratio than those of the other grasses. This is also the case in which it was pointed out in Part II (2, Fig. 2) that there was a phenomenal number of fungi (mainly *Penicillia*) in the soil. Possibly some constituent of brome residues, or its closely knit sod as already suggested (1), modifies the microbial balance in such a way as to reduce the supply of nitrates to the growing crop. Looking for further explanations of the position of timothy, we find its residues had the lowest percentage crude fibre and the narrowest ratio of crude fibre to nitrogen-free extract.

As pointed out in the first paper (1), the effects of timothy on succeeding crops did not turn out in this case in accordance with expectation. They are however in harmony with the facts deduced in the present paper. Both percentage and absolute quantity of nitrogen, and the percentage of crude fibre and ratio of this to readily fermentable carbohydrates in the residues of hay crops, appear to affect the availability of the nitrogen to succeeding crops.

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NITRIFICATION UNDER AND AFTER ALFALFA, BROME, TIMOTHY, AND WESTERN RYE GRASS

IV. OXIDATION-REDUCTION POTENTIALS AND CARBON DIOXIDE PRODUCTION[1]

By J. G. Malloch[2] and R. S. Young[3]

Abstract

Changes in the redox potentials of the soils and crop residues were caused mainly by the action of micro-organisms. No relation between the potential measurements and the yield or composition of the crops in the field could be demonstrated. All four forage plants have some fraction that was responsible for a marked drift in potential, but which disappeared early in the process of decomposition.

The greatest production of carbon dioxide occurred in soils under the hay crops, and it decreased with lapse of time after breaking. The production was greatest under and after alfalfa followed by brome. When the composition of the crop residues is taken into account, a relation between the carbon dioxide production and nitrate production can be demonstrated. The carbon dioxide production of decomposing residues decreases rapidly from a high initial value, giving further evidence of the presence of a readily decomposable constituent.

Introduction

In the first paper of this series (4) a comprehensive investigation of the sequence effects of alfalfa and certain grasses was outlined. Major emphasis has been placed on the effect of these crops on the rate of nitrification, but attention was also given to the general rate of decomposition, not only because it is important in itself but because there is evidence that nitrification is affected by the C:N ratio and by the decomposition of the non-nitrogenous constituents of the plants. Oxidation processes play a large part in this decomposition, and it was decided to make a special investigation of this phase of the problem. Both the intensity and the capacity factors received attention, the former by oxidation-reduction potential measurements and the latter by determination of carbon dioxide production.

Crop residues consisting of combined roots and stubble collected in the course of the main experiment, and samples of the top six inches of soil, were the experimental materials. The residues used came from four-year-old plots of alfalfa, brome, timothy, and western rye grass; the soils came also from the foregoing and from plots that had grown wheat for one year and three years following respectively three years and one year of these forage crops.

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Oxidation-reduction Potentials

The measurement of these potentials was undertaken to determine if there were any characteristic differences between the four forage crops used in the experiment and, if so, whether they were related to the field results. In the absence of any knowledge of the systems involved it could not be expected that any strict interpretation could be placed on the data. The potentials are simply relative measurements of an unspecified characteristic of the samples.

Method

The potentials were measured by means of a Leeds and Northrup Type K potentiometer and bright platinum electrodes of wire and foil. These were cleaned frequently by immersion in hot aqua regia. The potentials were found to be reproducible to within 0.1 mv.

The samples to be measured were placed in 100-cc. test tubes and stoppered. They were thoroughly shaken to incorporate the air or nitrogen before the first measurement of each series, but for subsequent measurements the electrodes were inserted with as little disturbance as possible.

Dried Roots and Stubble

Preliminary experiments had shown differences between the redox behaviour of fresh timothy residues and those of other crops. The initial experiments were therefore made with this kind of material. Water extracts were made from the residues and these were divided into two portions, one of which was buffered to pH 6.25, approximately the acidity of Edmonton loam on which the material was grown. Measurements of Eh were made at two-hour intervals up to 14 hr. and then at 24 hr. Only the initial and final Eh readings and any intermediate ones necessary to define the rate of change are given.

The readings for buffered and unbuffered extracts did not show any great differences and consequently only the results with buffered extracts are presented. The first experiment was repeated in the presence of nitrogen instead of in the presence of air. The gas was obtained from a commercial cylinder and, while the usual precautions to remove oxygen were taken, there probably were traces of oxygen present.

The next experiment was designed to study the effect of sterilization and subsequent inoculation with a suspension of soil micro-organisms. The sterilization was accomplished by use of a Pasteur-Chamberland filter.

The results of these experiments are shown together in Table I.

The untreated extracts show a marked change in potential in the presence of air. This change is very much reduced when the extracts are sterilized, although the initial level remains almost identical. On the addition of a suspension of soil micro-organisms the measurements were similar to those on untreated extracts, but the shift from positive to negative Eh took place several hours later, possibly owing to the induction period of the organisms.

TABLE I

EFFECT OF BACTERIAL ACTION ON THE REDOX POTENTIAL OF WATER EXTRACTS OF GROUND ROOTS AND STUBBLE

Treatment	Time, hr.	Eh, volts			
		Alfalfa	Brome	Timothy	Western rye
In presence of air					
Untreated	0	.55	.45	.48	.50
	8	.49	.38	.38	.39
	10	−.31	−.23	−.10	−.31
	24	−.33	−.33	−.32	−.33
Sterilized	0	.58	.46	.47	.50
	14	.55	.44	.44	.45
	24	.16	.15	.14	.20
Inoculated	0	.56	.46	.48	.51
	8	.47	.38	.38	.44
	10	.08	.25	.25	.29
	14	.04	.15	.20	.14
	24	−.33	−.32	−.29	−.32
In presence of nitrogen					
Untreated	0	.35	.35	.38	.35
	12	.35	.22	.25	.24
	14	.05	.09	.13	.15
	24	.03	.07	.10	.13
Sterilized	0	.35	.35	.35	.35
	24	.32	.32	.33	.33
Inoculated	0	.38	.38	.39	.38
	14	.32	.32	.29	.29
	24	.22	.24	.23	.21

It is clear that bacterial action is responsible for most of the change in potential, although there is a drift even in the sterile extracts. The immediate cause of the changes in potential was the disappearance of the dissolved oxygen. Shaking of the suspensions caused a return to the initial value. However, the changes can still be regarded as indicative of bacterial action since this is responsible for using up the oxygen supply.

The changes in the presence of nitrogen are much less marked and they begin from a lower initial level. The sterile extracts were quite stable, but as there was a slight drift when organisms were present, it seems that some bacterial action proceeded even when nitrogen was used. It is possible, however, that traces of oxygen were present. The measurements in the presence of air seem to be more informative and the use of nitrogen was discontinued.

The differences between the grasses are slight, particularly in the inoculated series, where the introduced flora was identical for all three. There is no evidence of differences that could be related to any effect the growing of these

grasses might have on subsequent crops. Alfalfa gives a higher initial value than the grasses and the change in potential takes place more rapidly. Bacterial action must have proceeded at a greater rate, and since this holds where a common inoculum was used it must be attributed to a difference in composition between the grasses and alfalfa.

Fermenting Roots and Stubble

The potentials of the crop material in process of decomposition are of more practical interest than those of fresh residues. The various crop residues were mixed with sand, brought to a moisture content equal to 50% of the water-holding capacity and allowed to undergo decomposition at 25° C. for varying periods of time. The potentials were measured in buffered water extracts. As with the fresh residues, the effect of adding suspensions of organisms to the water extracts after sterilization was tried. The results are given in Table II.

TABLE II

EFFECT OF FERMENTATION ON THE REDOX POTENTIALS OF WATER EXTRACTS OF MIXTURES OF SAND AND CROP RESIDUES

Sample	Time, hr.	Eh, volts			
		Alfalfa	Brome	Timothy	Western rye
After mixing					
Untreated	0	.49	.43	.41	.45
	8	.25	.15	.26	.30
	10	.08	−.33	−.13	.08
	12	−.06	−.33	−.18	−.01
	24	−.32	−.33	−.33	−.33
Sterilized and inoculated	0	.51	.46	.49	.54
	8	.11	.25	.30	.28
	10	−.33	−.05	.02	.05
	12	−.33	−.12	−.07	−.09
	24	−.33	−.34	−.33	−.33
After 3 weeks' fermentation					
Untreated	0	.52	.50	.50	.52
	14	.40	.42	.32	.41
	24	.28	.24	.20	.28
Sterilized and inoculated	0	.52	.53	.50	.50
	10	.22	.17	.09	.23
	14	.05	.00	.02	.10
	24	−.16	−.18	−.17	−.15
After 6 weeks' fermentation					
Untreated	0	.58	.59	.59	.58
	24	.53	.52	.51	.53
Sterilized and inoculated	0	.56	.57	.54	.55
	8	.50	.50	.48	.51
	24	.45	.44	.42	.49

In the series run directly after the preparation of the sand-residue mixtures there are marked changes in potentials in both parts of the experiment, and in this respect the results are similar to those obtained with residues alone. However, in this case there are differences between the grass residues, the change in brome being most rapid. As decomposition progresses the change in potential decreases until at six weeks the potentials are relatively stable over the 24-hr. period and all the crops are alike.

The samples were all shaken at the end of the 24-hr. measurement period to incorporate oxygen. The potentials of the extracts of freshly mixed sand and residues returned to the initial values, but the three- and six-weeks series underwent no change. With the freshly mixed samples bacterial action is rapid and the oxygen supply is a limiting factor. This does not hold for the series that had undergone partial decomposition. The difference can be explained by postulating the presence of a readily decomposable fraction in the fresh residues which disappears quite early in the process of decomposition. The values obtained after six weeks' fermentation might be expected to have the most practical significance, but in this case no differences exist between the crops.

Soils

To get more direct information on the potentials existing when the residues were decomposing under field conditions, soil samples were taken from plots growing their third crop of wheat following one year's growth of alfalfa and the grasses, from plots growing the first crop of wheat following three years' growth of the forage crops, and from the plots growing the four forage crops. The potential measurements were made on buffered soil suspensions in the presence of air. The data are given in Table III.

TABLE III

Redox potentials of soil suspensions

Soil	Time, hr.	Eh, volts			
		Alfalfa	Brome	Timothy	Western rye
1 year forage } 3 years wheat }	0	.55	.56	.53	.54
	24	.51	.51	.50	.51
3 years forage } 1 year wheat }	0	.53	.54	.54	.53
	24	.52	.52	.51	.51
4 years forage	0	.59	.55	.56	.54
	24	.45	.46	.47	.48

There are no essential differences between the soils growing wheat. Neither the previous crop nor the length of time it grew affect the potentials. The soils under the forage crops show a slight drift in potential and this can be attributed to the presence of the same transitory substance that caused the drift in the experiments with residues.

Some experiments were done in which residues were added to the soil and allowed to decompose. The results with extracts made after various periods of fermentation were as expected from previous experiments. There was a drift in potential in extracts made during the initial stages of decomposition, with slight differences in the rate between the different crops, but by the eleventh day the potentials for all samples became stable at 0.57 to 0.60 v.

The investigation failed to disclose any differences in the characteristics of the four forage crops that could be related to the sequence effects of these crops in the year in which the experiments were performed, and which are discussed in the first paper of the series (4).

Carbon Dioxide Production

Method

A review of the many methods for the determination of carbon dioxide production in soil described in the literature shows that the majority of investigators have concluded that aspiration over the soil gives a truer measure of microbiological activity than aspiration through the soil, the latter method producing an abnormal soil atmosphere. The former procedure was therefore adopted.

The apparatus and the method of determining the carbonate were essentially those used by Newton and Anderson (2). The blank for the entire determination, which includes errors arising from impurities in chemicals, leakages in the apparatus and any inefficiency in the towers used to wash the entering air free from carbon dioxide, was small and quite constant. In 11 determinations the value of the blank varied only from 2.28 mg. to 2.54 mg. of carbon dioxide and the appropriate corrections were made in the apparent carbon dioxide production of the experimental samples. The titration was checked by use of a solution containing sodium carbonate equivalent to 20.76 mg. of carbon dioxide. The values obtained in five trials varied from 20.64 mg. to 20.70 mg. with an average of 20.68 mg.

In the experiments 100-gm. portions of dry soil were weighed into a 300 cc. Erlenmeyer flask. When plant residues were added an amount corresponding to one gram of organic matter was incorporated. The soil was then brought to a moisture content equal to 50% of the water-holding capacity and the flasks were placed in an incubator at 25° C. The moisture content of the samples was maintained by addition of distilled water when necessary.

Three techniques for surface aspiration of the samples were tried: (i) the flasks were attached to the absorption towers continuously and aspirated for 20 min. at the end of a 24-hr. period: (ii) the flasks were attached continuously and were aspirated for 20 min. at 6, 12, and 24 hr.; (iii) the flasks were attached to the towers only for 20 min. aspiration at the end of a 24-hr. incubation. In parallel determinations the amounts of carbon dioxide found using the three techniques, were 81.2 mg., 85.2 mg. and 79.5 mg. respectively. The

last method gave somewhat lower results but the difference is not great and it was adopted because of its obvious convenience.

Soils

The plots were sampled by taking two six-inch borings from each of the four replicates and compositing. Duplicate determinations were made on these composites. The carbon dioxide produced during the first 24 hr. was determined and thereafter during the 24-hr. period following 3, 6, 9, and 12 days' incubation. The results reported in Table IV give the averages of the five determinations.

TABLE IV

Carbon dioxide production of soils growing wheat and forage crops

Crop	CO_2 produced in 24 hr. at 25° C., mg.			
	Alfalfa	Brome	Timothy	Western rye
Wheat, 3rd year after 1 yr. forage	3.8	3.0	3.1	3.1
Wheat, 1st year after 3 yr. forage	4.6	4.2	3.9	3.6
Forage, 4th yr.	6.4	8.1	4.9	4.7

The rate of decomposition, as indicated by the carbon dioxide production, is higher in the newly broken sods than it is in the plots that were broken two years previously. It is higher still in the plots growing forage crops. This is no doubt connected with the observation that the amount of crop residues did not accumulate as the sods grew older (4, p. 219). The explanation that in older sods "decomposition apparently proceeded at a greater rate than accretion by new growth" receives support from the high rate of carbon dioxide production.

Reference to the previous papers of this series shows that these results bear no direct relation to the yields obtained in the field (4) nor to the number of micro-organisms present at the time of sampling (1). As carbon dioxide production is a measure of decomposition, and nitrogen is presumably released from the residues at a rate proportional to this and to its relative abundance in the residues, the relation between carbon dioxide production and nitrate formation was investigated. Since it can be assumed that, in plots growing wheat for the first year following the plowing of three-year-old sods, the composition of the material decomposing in the soil is essentially the same as that of the residues from the previous crop, the data from these plots (3) can be used to test this relation. Root material far exceeds stubble in these residues and the composition of the former was taken as representative of the decomposing material. The calculated relative nitrate production was obtained by dividing the carbon dioxide production rate by the C : N ratio (3). This can be compared with the actual relative amounts produced in the soils, obtained by adding the amount of nitrogen absorbed by the wheat crop (4) to the amount remaining in the soil at harvest (1). No correction is necessary for the amount previously present, as nitrates were present only as a trace at

the time of plowing the forage sods. The two sets of figures were brought to a common basis by totalling each of them and expressing the individual results as percentages of the respective totals.

TABLE V

COMPARISON OF CALCULATED AND ACTUAL RELATIVE NITRATE PRODUCTION RATES

Crop	Ratio of carbonaceous to nitrogenous constituents	CO_2 production, mg.	Relative nitrate production rate	
			Calculated	Actual
Wheat after alfalfa	6.3	4.6	37	31
Wheat after brome	12.1	4.2	18	17
Wheat after timothy	8.6	3.9	23	26
Wheat after Western rye	8.0	3.6	23	26

The agreement between the relative rates of nitrate production actually found in the field and those calculated from the carbon dioxide production is surprisingly good, and, while the data are not extensive enough to constitute conclusive proof, a relation between carbon dioxide production and nitrate production is definitely indicated. The alfalfa gives the highest value in both calculated and observed nitrate production, and brome the lowest, with the other two grasses similar and intermediate. The carbon dioxide production was higher in soil after brome than after the other grasses but, because of the lower percentage of nitrogen present in the residues, more carbonaceous material had to be decomposed and the nitrate production was lower.

DECOMPOSING FORAGE CROP RESIDUES

A comparison of the susceptibility of the different residues to decomposition was attempted by adding these to portions of the same soil. The mixtures were then incubated at 25° C. for 35 days and the carbon dioxide production determined at intervals. In the results reported in Table VI the carbon dioxide production of the soil alone has been deducted and the figures given are for the gas produced from the residues only.

TABLE VI

PRODUCTION OF CARBON DIOXIDE FROM DECOMPOSING CROP RESIDUES AFTER SUCCESSIVE PERIODS OF INCUBATION AT 25° C.

Period of incubation, days	CO_2 produced in 24 hr., mg., from 1 gm. organic matter			
	Alfalfa	Brome	Timothy	Western rye
1	70.5	50.8	53.5	53.8
4	46.0	38.3	32.7	43.9
7	22.5	20.6	15.1	21.4
11	19.2	18.3	19.8	19.1
14	13.1	12.5	13.1	14.1
18	5.2	3.8	7.4	9.0
21	3.7	4.3	5.5	9.1
25	3.8	4.6	5.1	4.6
28	1.9	2.4	4.5	4.3
32	1.9	2.3	3.4	2.6
35	1.8	2.2	3.2	2.2

The initial rate is very high and the values do not drop for about four weeks to those found in the previous experiment with soil from the plots. During this period the readily decomposable fraction of the residues, to which we earlier attributed the drift in oxidation-reduction potentials, was presumably used up. The time necessary for the measurements to become relatively stable, indicating the disappearance of this fraction, is of the same order in both experiments.

At the end of the experimental period timothy was decomposing faster than the other crops, alfalfa being the slowest. There is no evidence that the depressive effect of timothy on subsequent crops, which is sometimes observed, is due to difficulty in decomposing the residues.

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THE EFFECT OF NUTRIENT SALTS IN ORGANIC MERCURIAL SEED DISINFECTANTS ON THE GERMINATION AND EARLY GROWTH OF WHEAT[1]

BY N. H. GRACE[2]

Abstract

Wheat seed was treated by dusting with a series of seed disinfectants containing 5% of ethyl mercuric halide (80 Br : 20 Cl) and three concentrations of each of KNO_3, KH_2PO_4, NH_4KHPO_4, and $Ca(H_2PO_4)_2 \cdot H_2O$. This method of applying nutrient salts failed to effect any general stimulation to germination or early growth. In one experiment with Marquis wheat, KNO_3 accelerated germination without increasing the final value, and all but KH_2PO_4 increased root weight three weeks after planting. In two subsequent experiments with only KNO_3 added to the mercurial, but with four varieties of wheat, conducted at different temperatures, no stimulation was observed.

Recent work on the treatment of plant cuttings and seeds with dusts containing a small amount of growth stimulating chemicals has suggested that other factors, such as nutrient salts, might be used effectively in the same manner (3, 4). Preliminary experiments on the use of nutrient salts in seed disinfectant dusts gave results that suggested slight stimulation to the germination and early growth of wheat. The present communication gives the results of a more detailed investigation in which several nutrient salts were used in an organic mercurial seed disinfectant.

Experimental

The mercurial dusts all contained 5% of ethyl mercuric halide* in a good grade of commercial talc, and were subjected to a grinding mix for about 15 hr. in a laboratory ball mill. Four nutrient salts, at three concentrations, were used with the organic mercurial. The salts were KNO_3, KH_2PO_4, $Ca(H_2PO_4)_2 \cdot H_2O$, and NH_4KHPO_4. The dusts with KH_2PO_4 contained 2, 5, and 10% of the salt. The amounts of KNO_3 added gave the same amounts of potassium as were contained in the dusts at 2, 5, and 10% of KH_2PO_4. Similarly, the amounts of the other two salts used gave the same phosphate concentration as was provided by KH_2PO_4 at its three levels of concentration.

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* *The ethyl mercuric halide used in these experiments was prepared by a method developed in the Division of Chemistry, National Research Laboratories, Ottawa, by Dr. A. Cambron. This procedure yielded a product consisting of 80% ethyl mercuric bromide and 20% ethyl mercuric chloride.*

Three experiments were carried out; in the first, 227-gm. samples of Marquis wheat were treated with the dusts at the rate of one-half ounce of dust per bushel of seed. Both seed and dust were placed in a glass bottle which was subjected to the action of a mechanical roller for a period of five minutes, a procedure which ensured thorough mixing. In the second and third experiments, 113.5-gm. samples of Dawson's Golden Chaff, Red Fife, Renown, and Reward wheats were treated at the rate of one-half ounce of dust per bushel of wheat with four dusts of the series. These dusts comprised organic mercury alone and organic mercury with the three levels of potassium nitrate.

The treated seeds were planted in sterilized soil in small 4 × 4 in. cardboard flats, 50 seeds to each flat, the unit in the experiments. In the first experiment the four salts were used at three concentrations, along with a control containing organic mercury alone, of which four flats were included in each replicate in order to provide averages of equal weight for all dosages and the control. The 16 flats receiving the various treatments were completely randomized in respect of position within each block, and there were 10 replicate blocks in all, which involved a total of 8000 seeds. In the second and third experiments there were also 10 blocks, each block being square and containing the varieties in compact sub-blocks of four units. Varietal sub-blocks were randomized as to position within main blocks, and the four treatments occurred at random within each of the four sub-blocks. In all three experiments the blocks were flanked by a row of flats containing untreated seed; this procedure reduced border effects.

In Experiment 1 the seed was planted November 30, 1938, in a greenhouse room maintained at approximately 55° F., and daily germination counts were taken as soon as germination was observed, which was eight days after planting. These daily counts were continued until germination was virtually complete. The plants were washed out of the soil 21 days after planting, held in an oven at 95° C. for two hours, and conditioned for one week in the laboratory. The exhausted seeds were picked off and the air-dry root and stem weights for the plants from 50 seeds were determined.

In Experiment 2, the seed was planted January 25, 1939, in a greenhouse room which ranged in temperature between 65 and 70° F. Daily germination counts and air-dry root and stem weights, 24 days after planting, were taken in the manner already described. In Experiment 3, the seed was planted February 22, 1939, in a greenhouse room maintained between 50 and 55° F. Daily germination counts were determined and also the air-dry weight of plants (stems and roots together) 33 days after planting.

In Experiment 1, the germination data were analysed statistically over the first three daily counts, the last five daily counts, and over all eight counts. In Experiments 2 and 3, germination rates were computed from the daily germination counts by the method of Bartlett (1); in addition, the final germination count was also considered. All the data were subjected to analyses of variance.

Results

Experiment 1

Germination data over the first three daily counts, the air-dry weight of roots from 50 seeds, and the analyses of variance of the responses to treatment are given in Tables I, II, and III, respectively. Potassium nitrate increased germination over the first three daily counts, but none of the levels of KH_2PO_4, $Ca(H_2PO_4)_2$, or NH_4KHPO_4 differed significantly from the control. Stimulation from potassium nitrate treatment, 49% germination against 38.2% for the control on the third day, may be attributed to the two highest levels used, namely, 3.7 and 7.4% of salt in the mercurial dust. It is apparent from Table III that stimulation of germination, under the conditions of this experi-

TABLE I

GERMINATION OVER FIRST THREE DAYS OF MARQUIS WHEAT TREATED WITH NUTRIENT SALTS IN AN ORGANIC MERCURIAL SEED DISINFECTANT

(Experiment 1)

Nutrient salt	Transformed data[1]					Germination on the third daily count, means by salts[2], %
	Control (organic mercurial alone)	Relative concentration of nutrient salt in mercurial disinfectant			Mean for all three nutrient salt concentrations	
		1	2.5	5		
KNO_3	2.346	2.705	2.807	3.086	2.866	49.0
$Ca(H_2PO_4)_2$	2.346	2.332	2.583	2.593	2.503	38.2
KH_2PO_4	2.346	2.238	2.482	2.083	2.268	34.6
NH_4KHPO_4	2.346	2.077	2.246	1.987	2.103	30.4[2]

Necessary difference, 5% level of significance, between treatments, 0.562; treatments and control, 0.444; treatment mean and control, 0.304.

[1] *Data transformed to $\sqrt{x + \frac{1}{2}}$ basis (2) for analysis of variance.*

[2] *Mean germination for the control groups, with organic mercury only, on the third daily count was 38.2%.*

TABLE II

AIR-DRY WEIGHT (GRAMS) OF ROOTS FROM PLANTS GROWN IN 21 DAYS FROM 50 SEEDS, TREATED WITH NUTRIENT SALTS IN AN ORGANIC MERCURIAL SEED DISINFECTANT

(Experiment 1)

Nutrient salt	Control (organic mercurial alone)	Relative concentration of nutrient salt in mercurial disinfectant			Mean for all three nutrient salt concentrations
		1	2.5	5	
KNO_3	0.228	0.242	0.263	0.238	0.248
$Ca(H_2PO_4)_2$	0.228	0.231	0.257	0.245	0.244
KH_2PO_4	0.228	0.235	0.233	0.226	0.231
NH_4KHPO_4	0.228	0.242	0.240	0.245	0.242

Necessary difference, 5% level of significance, between treatments, 0.013; treatments and control, 0.010; treatment mean and control, 0.007.

TABLE III

ANALYSIS OF VARIANCE OF RESPONSE OF MARQUIS SEED TREATED WITH NUTRIENT SALTS IN AN ORGANIC MERCURIAL DUST DISINFECTANT

(Experiment 1)

Source of variance	D.f.	Mean square				
		Germination over			Air-dry weights ($\times 10^4$)	
		First 3 days	Last 5 days	All 8 days	Stems	Roots
Replicates	9	9.23**	37.41	236.1**	17.71**	2.03**
Treatments	12	3.11**	39.97	117.0**	3.31	1.44**
Error	138	1.19	28.83	42.5	2.24	0.60

** *Exceeds mean square error, 1% level of significance.*

ment, was confined to the first three days on which counts were made and did not extend to the subsequent five daily germination counts.

While nutrient salt treatment failed to increase significantly the weight of stems, there were some significant increases in the air-dry weight of roots. Considering the means for all three levels of the four salts, we note that all the salts but KH_2PO_4 increased root weight. All three concentrations of potassium nitrate effected an increase in root weight with the intermediate concentration having the greatest activity; the root weight fell significantly on further increase of the salt in the dust. The two highest concentrations of $Ca(H_2PO_4)_2$ gave increased root weights, but, while the weights fail to differ significantly, a falling off is suggested at the highest level. All three treatments with NH_4KHPO_4 gave increased root weights; however, the response is essentially the same at all concentrations. No significant variation in root weight was effected by KH_2PO_4 treatments.

The results suggest that the increased root weights obtained with KNO_3 may be attributed to the presence of nitrate, since KH_2PO_4 had no effect, and dusts of these two salts contained identical amounts of potassium. Similarly, the activity of the other two salts, having the same amount of phosphate as KH_2PO_4, appears to be related to the presence of calcium and ammonium nitrogen.

Since treatments with potassium nitrate gave the greatest effects on root weights, and the only stimulation to germination in the experiment, succeeding experiments were confined to this one salt. However, four varieties of wheat were employed in an effort to investigate the possibility of differential effects of treatment upon varieties.

Experiment 2

The analyses of variance for the data are given in Table IV. Highly significant varietal differences were shown for all of the properties measured. The only significant treatment effect was upon germination and this was in a negative direction. There were no interactions between varieties and treatments.

TABLE IV

ANALYSIS OF VARIANCE OF RESPONSE OF FOUR VARIETIES OF WHEAT SEED TREATED WITH POTASSIUM NITRATE IN AN ORGANIC MERCURIAL SEED DISINFECTANT

(Experiment 2)

Source of variance	D.f.	Mean square			
		Germination rate	Final germination count	Air-dry weight	
				Stems	Roots
Replicates	9	0.0259***	1.692	0.0382***	0.0132***
Varieties	3	0.4482***	43.875***	0.6885***	0.2470***
Interaction					
Varieties × replicates (error (a))	27	0.0038	3.229	0.0045	0.0015
Treatments	3	0.0144***	1.842	0.0030	0.0006
Interaction					
Varieties × treatment	9	0.0021	1.714	0.0022	0.0006
Error (b)	108	0.0021	1.713	0.0040	0.0011

*** *Exceeds mean square error, 0.1% level of significance.*

In Tables V and VI are given data for the different varieties and effects of potassium nitrate treatment on the germination rate. The effect of treatment with potassium nitrate, averaged over all four varieties, was a significant reduction in the germination rate when 7.43% of the salt was present in the organic mercurial disinfectant. Neither the 1.49% nor the 3.71% levels of potassium nitrate differed significantly from the control, though a decrease in rate was suggested by the higher of these two concentrations.

Absence of stimulation to germination, or stem and root weights, suggested that the higher temperature under which growth occurred (about 68° as compared with about 55° F. in Experiment 1) might have had an effect on

TABLE V

AVERAGE RESULTS, ALL TREATMENTS, WITH WHEAT PLANTS GROWN IN 24 DAYS FROM 50 SEEDS OF EACH OF FOUR VARIETIES

(Experiment 2)

Variety	Germination rate	Final germination count of 50 seeds planted	Air-dry weight, gm.	
			Stems	Roots
Dawson's Golden Chaff	0.828	49.9	0.95	0.41
Red Fife	0.682	48.8	0.63	0.23
Renown	0.743	47.8	0.80	0.33
Reward	0.576	47.5	0.76	0.27
Necessary difference, 5% level of significance	0.028	0.8	0.03	0.02

TABLE VI

EFFECT OF POTASSIUM NITRATE IN AN ORGANIC MERCURIAL SEED DISINFECTANT ON GERMINATION RATES OF FOUR VARIETIES OF WHEAT

(Experiment 2)

	Concentration of salt in disinfectant dust, %				Necessary difference, 5% level of significance
	0	1.49	3.71	7.43	
Rate	0.720	0.720	0.709	0.680	0.020

the results. In consequence, Experiment 3 was a repetition of Experiment 2, but with temperature held at about 55° F.

Experiment 3

Tables VII and VIII give the analyses of variance of the responses and the data for germination rates, final germination, and air-dry plant weights for four varieties of wheat. It is apparent that the only significant feature of the experiment was the difference between varieties. There were no treatment effects nor any interactions between treatments and varieties. The four varieties fell into the same order as they did in Experiment 2. In view of these results, absence of stimulation from potassium nitrate treatment in Experiment 2 cannot be attributed to the higher temperature under which growth occurred.

The addition of nutrient salts, along with an organic mercurial in a seed disinfectant dust, thus failed to effect any general stimulation to the germination and early growth of wheat. Owing to the season of the year, the two

TABLE VII

ANALYSIS OF VARIANCE OF RESPONSE OF FOUR VARIETIES OF WHEAT SEED TREATED WITH POTASSIUM NITRATE IN AN ORGANIC MERCURIAL SEED DISINFECTANT

(Experiment 3)

Source of variance	D.f.	Mean square		
		Germination rate	Final germination count	Air-dry weight of plants
Replicates	9	0.0024	6.334	0.3685***
Varieties	3	0.2061***	73.990***	3.1114***
Interaction				
Varieties × replicates (error (a))	27	0.0014	3.036	0.0494
Treatments	3	0.0007	3.556	0.0036
Interaction				
Varieties × treatment	9	0.0010	1.984	0.0288
Error (b)	108	0.0009	2.784	0.0172

*** *Exceeds mean square error, 0.1% level of significance.*

TABLE VIII

AVERAGE RESULTS, ALL TREATMENTS, WITH WHEAT PLANTS GROWN IN 33 DAYS FROM 50 SEEDS OF EACH OF FOUR VARIETIES

(Experiment 3)

Variety	Germination rate	Final germination count of 50 seeds planted	Air-dry weight of plants from 50 seeds, gm.
Dawson's Golden Chaff	0.682	49.4	2.34
Red Fife	0.584	48.0	1.68
Renown	0.622	46.6	2.00
Reward	0.511	46.5	1.88
Necessary difference, 5% level of significance	0.017	0.8	0.10

experiments with potassium nitrate were grown under a greater intensity of light than the first experiment, which showed some significant stimulation. Light effects may accordingly have a bearing on the results obtained. Thus, there is an indication that there are growth conditions under which nutrient salts may cause stimulation to the early germination and growth of wheat, although these conditions are not such as would be expected to occur in the field.

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ACTION OF MICRO-ORGANISMS ON FAT*

II. OBSERVATIONS OF UNINOCULATED GLOBULES OF TRIGLYCERIDES AND FATTY ACIDS AND OF BUTTERFAT IN AN AGAR MEDIUM[1]

By C. H. Castell[2] and E. H. Garrard[3]

Abstract

With the object of discovering the significance of the "opaqueness" surrounding so-called lipolytic colonies on fat-emulsion agar plates, detailed observations have been made on uninoculated globules of butterfat, pure triglycerides, fatty acids, and mixtures of fats and fatty acids. The following observations have been made:

At room temperature, sooner or later, fat crystals form in all butterfat globules. The rapidity with which crystals form and their general conformation and texture are capable of considerable variation. This depends upon the previous history of the butterfat, temperature, and the physical and chemical characteristics of the agar medium in which the fat is suspended.

Sodium chloride, among other substances, brings about rapid changes in the form of the globules and the texture of the crystals.

The crystals of pure fats suspended in agar are quite unlike the crystals in globules of butterfat. When these same fats are suspended in some form of oily matrix this difference disappears.

The chief difference between pure fats and fatty acids, when suspended in oil, is that there is a tendency for the fatty acids to crystallize at the fat-agar interface, while the fats do not. The lower, solid fatty acids often protrude out into the agar medium.

As well as copper sulphate and Nile blue sulphate, a large number of other dyes have been used to differentiate fatty acids from fats.

It appears probable that the phenomenon of opaqueness in the fat globules surrounding lipolytic colonies in fat-emulsion agar plates is owing, at least in part, to the rapid formation of fat crystals, as well as to the formation of fatty acids.

Introduction

In the use of oil-emulsion agars for the identification of lipolytic bacteria, two types of observations are made. An indicator, such as Nile blue sulphate, may be incorporated into the medium, and lipolysis indicated by changes in the colour of this dye; and lipolysis may be indicated by physical changes occurring in the individual fat globules and observed as either a clear zone or an opaque area surrounding the colony in question. In a previous paper (3) examination was made into the significance which can be attached to the colour changes of certain dyes used in this manner. This present investigation will deal with the physical changes occurring in the individual fat globules.

Choice of Fatty Materials

There are two general types of fats used in these tests, namely, simple triglycerides and natural fats, such as cottonseed oil or butterfat. Anderson (1) recommended tributyrin in nutrient agar and used this medium for examining the microbial content of milk, cream, water, and sewage. Collins

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* *The first paper in this series appeared in the Iowa State Coll. J. Sci. 4 : 313-328. 1939.*

and Hammer (4) have shown that hydrolysis by bacteria of simple triglycerides was more difficult as the molecular weight increased. Long and Hammer (5) found that certain cultures commonly regarded as non-lipolytic would bring about reactions on plates containing either dispersed tripropionin or tributyrin, similar to the reaction brought about by bacteria capable of hydrolysing fats.

These observations show clearly that the action of an organism in hydrolysing or not hydrolysing one specific triglyceride does not necessarily indicate its action on others. Although the fats of lower molecular weights may give the largest number of positive tests with a group of cultures, such information might be of little value where applied to natural fats. Even with fats containing propionic and butyric acid radicals, it is unlikely that appreciable amounts will be in the form of pure triglycerides.

For these reasons it seems apparent that no one fatty material can be used universally for this purpose. As this series of papers deals as closely as possible with the action of bacteria on the fat of milk and milk products, butterfat will be the "natural" fat chiefly considered.

Microscopical examination of individual globules in the "opaque areas" surrounding a typical lipolytic colony growing in a butterfat agar emulsion medium, shows the opaqueness to be due to the formation of crystalline material. The use of polaroid discs makes this still more evident. Efforts will be made to discover the nature of these crystals. They may be fats, fatty acids, combinations of the two, or some other by-products of fat spoilage. Until this is known, the real significance of these opaque areas remains a question of doubt.

Experimental

Two series of observations have been made. The first deals with the globules formed from triglycerides and fatty acids singly and in various combinations. The second deals with globules of butterfat under various conditions. The results are then compared.

Observations on Globules of Triglycerides and Fatty Acids

These experiments were carried out with the hope of finding out whether the fats and fatty acids could be distinguished from each other under conditions comparable to the fat-emulsion-test media used for detecting lipolysis.

To 1% sterile melted agar, 3% of the fat, fatty acid, or fatty-acid–fat mixture was added. These were autoclaved at 10 lb. pressure for 10 min., and then after vigorous shaking, poured into sterile Petri dishes. They were incubated at 25° C. The following materials were used:

Triolein; tributyrin; tricaprin; tricaproin; trilaurin; trimyristin; tripalmitin; tristearin.

Triolein + trilaurin; triolein + trimyristin; triolein + tripalmitin; triolein + tristearin; triolein + tripalmitin + trilaurin; triolein + tripalmitin + trimyristin; triolein + tripalmitin + tristearin; triolein + tripalmitin + tristearin + trilaurin + trimyristin.

Oleic acid; palmitic acid; stearic acid.

Triolein + butyric acid; triolein + caproic acid; triolein + caprylic acid; triolein + lauric acid; triolein + myristic acid; triolein + palmitic acid; triolein + stearic acid; triolein + oleic acid; triolein + palmitic + lauric acids; triolein + palmitic + myristic acids; triolein + palmitic + lauric + myristic + stearic acids; triolein + palmitic acid + palmitin; triolein + lauric acid + palmitin; triolein + myristic acid + palmitin.

Added to these observations were others in which the triolein was replaced by paraffin oil, and some in which various dyes were added to the media. Details were noted and photomicrographs were made from each plate. Because of the unavoidable length of these descriptions only certain of the more important ones will be recorded in this paper.

Individual Triglycerides

There were no crystal formations in any of the globules of the four liquid fats, tributyrin, tricaproin, tricaprylin, and tricaprin. There were, however, slight differences between the globules of these fats. Those of the tributyrin sank to the bottom of the agar; those of the tricaproin and tricaprylin had a greater tendency to rise and spread out on the surface of the agar. Of the four, tricaprin had the greatest tendency to remain dispersed as small spherical globules in the agar. The globules of each of these four fats had spherical droplets within them. Many of these droplets appeared relatively unstable, disappearing when exposed to air or bright light.

Triolein differs from the fats described above in having an excessively large number of the colourless spherical droplets within the globules. These droplets in the surface globules are at first very unstable. When the cover of the Petri dish has been removed they suddenly disappear. Under the microscope this change appears to be one of dissolving rather than bursting. Several minutes after the lid of the Petri dish has been replaced, similar but very minute droplets may form again. The point of a hot needle against the edge of a surface globule causes the instant formation of countless numbers of these small spheres. When the Petri dishes have been incubated a week or more, there is a decided increase in the number and stability of these droplets. The addition of certain substances, such as Nile blue sulphate, methylene blue, or sodium chloride, reduces the number of these bodies.

Globules of the solid fats were at first spherical and homogeneous. On cooling, they crystallized and lost their spherical shape. As can be seen in the illustrations (Figs. 3 and 20), there is very little similarity between these and the crystal formations observed in normal butterfat.

Fat Mixtures

About 50% of the fats in butter are liquid at room temperature. This means that as the higher fats crystallize they do so in a matrix of oil. For this reason a series of plates was made in which the higher fats were mixed with triolein or paraffin oil. Under these conditions the fats crystallized in forms quite different from those in the agar alone. Tripalmitin and tristearin, and more especially tripalmitin with lesser amounts of tristearin, trimyristin

and trilaurin, produced crystal formations very similar to those observed in the butterfat (Figs. 21 to 23). It was always observed that crystals were never found in the agar outside of, or protruding from, the oily matrix (i.e., the globules of paraffin or triolein). In many cases the crystals were formed in the *centre* of the surface globules, rather than at the agar interface.

Although the solid fats showed considerable variation as they crystallized in the globules of triolein, there were sufficient characteristic differences to differentiate each fat microscopically. Tristearin and tripalmitin in globules of triolein frequently form crystal formations that are very similar, and in this form they are similar to the crystals that are first observed in globules of butterfat. These are shown in Fig. 24. They begin as a more or less irregularly circular mass of feathery or fern-like crystals radiating from a central point. In many ways they suggest the microscopical picture of a young actinomycete or mould colony. Later this may grow into such a compact mass that it is only with difficulty that the individual crystals can be seen. Trimyristin and trilaurin differ from tristearin and tripalmitin in having larger and coarser crystals and in forming crystal masses with much less symmetrical outline (Figs. 25 to 30).

Fatty Acids

At first, the crystals of the fatty acids forming in the globules of oil appeared similar to the corresponding fats. They are also subject to considerable variation. One noticeable difference is the tendency of the acids to crystallize at the fat agar interface (Figs. 31, 32 and 35) (i.e., on the outside of submerged globules and around the circumference of surface globules). With the softer acids the crystals frequently start at the surface and grow out into the agar (Fig. 31). This never occurs with the fats. Another difference between the crystals of fats and fatty acids is that the acids tend to form into a less compact mass. Frequently they produce separate needle-like crystals, or groups arranged in a fan-like fashion (Fig. 36). These characteristics are not invariable. Under some circumstances the acid crystals form completely within the fat, and many observations were made, especially with stearic and palmitic acids, where the crystals were formed into compact little masses, not unlike one of the forms assumed by tristearin. However, in any one plate there was always sufficient variation in crystal formation to enable even an inexperienced observer to differentiate between fats and fatty acids, and in most cases, to recognize the individual fats or fatty acids.

It was frequently observed that as the molecular weight of the acids decreased in these fat-acid mixtures, there was a greater tendency for the globules to unite and rise to the surface. Even on the surface itself, with lauric acid, there were large areas where the globules were grouped together leaving other areas free from globules altogether. This was much less noticeable with stearic or palmitic acids. But when lauric acid was added to either palmitin or palmitic acids (in triolein), this apparent increase in surface tension was evident.

THE ACTION OF COPPER SULPHATE AND OTHER INDICATORS IN DIFFERENTIATING GLOBULES OF FATS AND FATTY ACIDS AND THE REACTION OF THESE REAGENTS TO GLOBULES OF BUTTERFAT

Copper sulphate (6) Nile blue sulphate (2) methylene blue (3) and other indicators have been used in oil emulsion agars to indicate the production of fatty acids. Experiments were conducted with these indicators on uninoculated globules of triglycerides, fatty acids, and butterfat, under conditions similar to those in the oil-emulsion agar tests. The object was to test the ability of these reagents to indicate the presence of individual fatty acids, and then to compare this with their reaction on the globules of butterfat.

Plates were prepared and poured in the usual manner. Nile blue sulphate and methylene blue were added as the medium was being prepared. The saturated copper sulphate solution was flooded over the surface of the agar after the plates had been poured and hardened. The materials tested were: Stearic, palmitic, myristic, lauric, caproic, caprylic, and oleic acids and their corresponding triglycerides.

Copper sulphate: None of the fats showed any change. All the fatty acids ultimately turned blue. The liquid acids became blue on contact with the salt solution; myristic and lauric acids were doubtfully blue after 20 min. at room temperature, and definitely blue at the end of an hour; the stearic and palmitic acids were colourless at 20 min. but coloured at the end of an hour.

Nile blue sulphate: The liquid fats were coloured various shades of pink, rose, and red; the solid fats were less deeply stained and the colours were changed to shades of brownish red with tinges of purple as the melting point of the fat increased. The liquid fatty acids stained deep blue or shades of purplish blue; the solid fats stained less deeply, but still had sufficient blue colouring to enable them to be easily differentiated from the corresponding fats. These observations agree with those of Collins and Hammer (4), who have had extensive experience with this dye.

Methylene blue: None of the fats were stained; the liquid acids were blue; lauric acid was pale blue; myristic, palmitic, and stearic acids showed very little colouring.

When the temperature was raised above their melting points all the fatty acids produced instantaneous and intense colour reactions with each of these reagents.

Other indicators: Similar tests were made on caproic and caprylic acids and their corresponding triglycerides, with aqueous solutions of the following dyes: malachite green, methyl violet, basic fuchsin, neutral red, rose bengal, china blue, toluidine blue, gentian violet, saffranine O, rose-aniline hydrochloride, eosin, brilliant green, phenol-indo 2 : 6-dichlorophenol, and *p*-aminodimethylaniline monohydrochloride. In each instance the fats remained uncoloured and the acids were deeply stained. When myristic and stearic acids were added to a 1 to 1000 aqueous solution of these dyes and the temperature was raised above the melting point of the acids, they became coloured. (It is

of interest to note that all these dyes that stain the fatty acids appear to be oxidation-reduction indicators. The addition of a weak solution of sodium hydrosulphite or other reducing agent renders them colourless, and the addition of hydrogen peroxide restores the colour.)

Observations on changes occurring in uninoculated globules of butterfat

Unless otherwise stated, the butterfat used throughout these experiments was made from fresh, pasteurized, sweet cream; it was unsalted and the curd was removed by shaking the butter in warm water and separating the fat in a separatory funnel. The basic agar medium was prepared by adding 1% standardized granular Bacto agar to 1 litre of distilled water. The amount of fat used was 3% by volume. Unless otherwise stated, the medium was autoclaved at 15 lb. pressure for 15 min. Immediately before pouring the plates, flasks were vigorously shaken in order to disperse the fat in the form of small globules throughout the medium.

Observations, under varying conditions, demonstrate the fact that there are three or four main characteristic physical changes which may occur in uninoculated globules of butterfat. As these form a basis for further descriptions and may be significant when related to enzyme activity, it is necessary to give a brief description of them at the beginning.

A. Crystal Masses

When the plates are first poured, the globules of butterfat appear under the microscope as homogeneous, liquid, or semi-liquid bodies (Fig. 1). As the fat cools, crystals appear in the globules (Fig. 2). These crystals are subject to great variation, both in the form they assume and the rate at which they form. If the plates are incubated at 25° C., at the end of two or three days almost every globule will contain one or more large masses of feathery crystals which radiate from a central point (Fig. 3). These usually form first at the surface of the globule, appearing mainly in one plane; later they grow down into the fat, forming dense feathery crystal masses. After the formation of these primary crystal masses, either within them or forming a border around them, crystalline material of a different appearance may be formed. It appears as a softer wax-like material, with little or no indication of the feather-like crystal formation. That the melting temperature is one of the chief differences between these types of crystals, can be demonstrated by observing their action through a series of temperature changes (Figs. 15 and 16). The waxy-looking material melts at a lower temperature. Both types rotate polarized light. Given sufficient time, some form of the primary crystal masses invariably forms in globules of butterfat. The second type is observed less frequently and only after a longer incubation period. One characteristic was constant to all these crystal formations in the globules of butterfat. They never protruded out of the fat into the surrounding medium.

In all probability, the crystal masses described above are similar to those that Ritter (6) described as "hedgehog-like crystals" in his observations on cooked butter.

B. Submerged and Surface Globules

Apart from the temperature at which the plates are poured, and the vigour with which the medium is shaken, there are other factors that greatly influence the dispersion of the fat in the agar. The globules may remain small and spherical, with those near the surface completely or almost completely submerged in the medium; or they may have a tendency to unite, to rise to the surface, and to spread out on the top of the agar (Figs. 18 and 19). This may be owing to the formation of thin protecting films of phospholipoids or protein material around the individual globules. When the butterfat is fresh there is a greater tendency for it to remain dispersed as fine globules. The addition of even very small percentages of sodium chloride brings the globules to the surface.

This difference (whether the globules are dispersed in the medium or mainly at the surface) becomes more significant when bacterial action is considered. There are some species that appear to be more active in attacking fat when it is exposed to the air.

C. Droplets

Within the fat globules there are frequently found large numbers of small colourless spherical bodies (Figs. 9 and 12). They are associated with the liquid portions of the fat and are very abundant in globules of pure triolein. At times it is difficult to decide whether these are liquid or gaseous in nature or whether they are always the same substance. Occasionally they have been stained blue with both Nile blue sulphate and methylene blue. They vary a great deal in size, and under some conditions appear to be very unstable. Similar looking droplets appear in globules pierced by a portion of mould mycelium. In this latter instance they are apparently water droplets resulting from respiration.

Closer observation shows another type of droplet frequently found in butterfat. In fresh butterfat these droplets are darker in colour than the fat in which they are suspended. They are much smaller and more regular in size than the colourless droplets mentioned above. In size they are similar to the smaller fat globules of normal milk. Large globules may contain as many as a hundred of these bodies; smaller ones may contain none, one, or more. Careful examination shows that similar bodies are occasionally found in the agar away from the fat globules, but never in agar not containing the butterfat. At reduced temperatures these bodies crystallize and lose their spherical shape. All attempts to photograph these smaller types of droplets met with failure.

Effect of Temperature on Globules of Butterfat

Many observations were made on globules of butterfat at temperatures ranging from 5° C. (41° F.) to 37° C. (98° F.). These may be summarized as follows: Other things being equal, decrease in temperature brings about faster, finer, and more complete crystallization, with less tendency towards differentiation of crystal types. At any one temperature there appears to be

a maximum amount of crystalline material formed, but the speed at which it forms varies with the past history of the butter sample as well as with the physical and chemical characteristics of the medium in which the fat is dispersed.

At a temperature of 25° C. (77° F.) approximately 30 to 50% of the globule ultimately becomes crystalline and the crystals are relatively large and coarse. At 5° C. (41° F.) the whole globule appears to become a solid mass of crystalline material. As the crystals are finer at this temperature, there is much less distortion of the shape of the globules than when they are allowed to crystallize at higher temperatures. When samples were suddenly cooled, and after crystallization incubated for long periods at room temperature, they never became as coarse or irregular as similar samples incubated at room temperature without the pre-cooling (Fig. 17).

Addition of Various Substances to the Agar Medium

In order to encourage bacterial growth and to adjust the pH, etc., a considerable number of different materials may be added to the various fat-emulsion agars used for detecting bacterial lipase. Little consideration has been given to the effect of these added substances on the mechanism of the tests for fat spoilage, or the changes they bring about in the fat itself. It will be shown in experiments to be reported later that very small amounts of sodium chloride enable an organism, otherwise considered non-lipolytic, to bring about changes similar to a lipase-positive organism.

In this section a few observations will be made on the action of some common substances on globules of butterfat.

Sodium Chloride—Sodium chloride, even in relatively small amounts (0.5% and less) has a marked effect on the butterfat in an agar emulsion. The globules are changed in such a way that instead of remaining submerged or partly submerged in the agar, they tend to rise to the surface and spread out. The crystal masses formed in these surface globules are relatively large and very coarse (Figs. 3 and 5); differentiation of crystal types is marked; and the production of the larger colourless droplets associated with the more liquid portion of normal butterfat globules is either greatly reduced or entirely absent. On further incubation the globules tend to become very irregular (Fig. 6).

Incubation of the plates in the dark at room temperature for several weeks brings out still further differences between salted and unsalted plates. The surface globules in the medium to which the salt has been added tend to contract and leave empty a portion of the mould which they originally made in the agar (Fig. 4). In many cases the structural make-up of the globules appears to assume a definite plan. The crystal masses form dense areas around the outer surface and bottom of the globules, giving them the appearance of many little craters (Fig. 7). The central area, which at first appears empty, contains the liquid and semi-liquid portions of the globule. And this in turn, by the addition of dyes, can be shown to contain a more or less spherical, colourless, central body (Fig. 8).

As most of this crater-like formation of the globules when salt is added occurs several weeks after the plates have been poured, it has little interest as far as it pertains to the immediate use of such plates for bacteriological tests. However, it is of interest in showing the peculiar action of salt on the fat globules, and it is of further interest when it is realized that the enzymes of certain types of micro-organisms bring about similar changes in the fat after a few days' incubation.

Compared to the unsalted butter, the addition of salt hastens the bleaching of the globules and the production of tallowy odour from the plates. So far, it has been difficult to determine whether this accelerated oxidation is owing only to the fact that on the salted plates the globules are more exposed to the atmosphere, or whether some other factor is involved.

Other Substances—The addition of 5% normal sodium hydroxide, sodium bisulphite, peptone, and many other substances, has an effect similar to sodium chloride in bringing about some change (probably in surface tension) which caused the globules to rise to the surface of the medium and spread out. Further changes varied considerably with the amount and the nature of the substance added.

Variations in pH of the medium, brought about by the addition of either sodium hydroxide or lactic acid, caused changes in size and texture of the crystals and in the form of the globules (Figs. 10, 11, and 12). The variations are greater as the value increases or decreases from somewhere near neutrality. At the lower pH range, the crystal masses are large and coarse; slightly above neutrality, from pH 7.2 to 8.5, they are less and finer.

Many other experiments were tried, such as thorough and repeated washing of the butter, prolonged shaking as an emulsion in a vibration machine (the results of which are shown in Fig. 14), heat treatments, and the effect of age of the butter (Fig. 13). All brought about changes in the globules that were fairly constant.

Discussion

The observations described have indicated that the crystals formed from the solid triglycerides in a matrix of oil are very similar to those found in globules of butterfat. They are quite different when they crystallize in agar alone. Although the crystalline structures of these fats are capable of considerable variation, there are characteristic differences by which the higher fats, tristearin and tripalmitin, can be readily differentiated from the softer fats, trimyristin and trilaurin, when observed in these microscopic oil globules. In a similar way the corresponding fatty acids can be distinguished.

It is not always possible to differentiate between the crystals of fats and fatty acids as they form in these globules. There are, however, some characteristic differences that frequently enable almost instant differentiation. These characteristics become more pronounced as the number of carbon atoms in the acids decrease. The fat crystals tend to form wholly within the oil globule. The fatty acids tend to crystallize around the surface of the oil

globules, and with the softer fats they grow out into the agar. Such orientation is in agreement with our knowledge of the affinity between water and one end of the fatty acid molecule; this relation does not exist with the triglycerides.

Another frequent difference is that the fats always form crystal masses in which the crystals radiate out from a central point. Fatty acid crystals frequently form singly or in fan-shaped masses, or in other less compact forms.

Copper sulphate, Nile blue sulphate, methylene blue, and many other indicators gave colour reactions with fatty acids, which do not occur with either the pure triglycerides or globules prepared from butterfat.

Globules of butterfat dispersed in an agar medium, which are at first homogeneous, have been observed to go through a series of easily observed changes. These consist mainly of the formation of various types of crystal masses within the globules, the appearance of minute spherical bodies in the more liquid portion of the fat, and finally what appear to be changes in surface tension, determining whether the globules remain small and spherical, dispersed throughout the agar, or partially unite to form large flat globules on the surface of the medium.

Sudden chilling of the globules at low temperature brings about the complete crystallization of the fatty material; the crystals are very fine and the globules do not lose their spherical shape. When crystallization is slower the crystals are larger and coarser, and the globules lose their spherical shape owing to distortion by these crystals. Slow crystallization brings about differentiation of crystalline material into at least two types. The last formed is the first to melt as the temperature rises.

Globules of fresh unheated butterfat in pure agar to which no other material has been added remain "normal" for the greatest length of time. The addition of many other substances, washing, heating, severe shaking, and ageing of the butter, all tend to hasten crystallization of the fat and bring about other changes in the globules.

By comparing the crystals that form in globules of butterfat with those of the pure triglycerides formed under similar conditions, there is every indication that the crystals in the butterfat are those of the higher melting-point fats. The amount of these crystals forming in the butterfat agrees approximately with the known percentage of the higher fats in butter. It is also apparent that the "opaqueness" in fat globules following bacterial activity may be owing to the rapid crystallization of fats as well as to the formation of fatty acids. Observations have been made on differences in the formation of fat and fatty acid crystals. These, together with the colour reactions of the fatty acids in the presence of copper sulphate, or any one of a large number of oxidation-reduction indicators, will enable the differentiation of fat crystals from those of fatty acids in globules that have been subjected to bacterial activity.

Apart from their application to further studies on the action of micro-organisms on fats, some of the observations recorded here appear to have an application in other fields. The texture of butter and other fatty foods is determined to a considerable extent by crystal structure. Fats in globular form afford a splendid opportunity for observing and photographing crystals, and changes occurring in crystals. They can be handled with facility and easily subjected to variation in environment. An example of the practical use of such observations is seen in the rapid pre-cooling of the butterfat globules incubated at room temperature. They are more uniform and contain finer crystals than those incubated at the same temperature without the short pre-cooling. This may have its counterpart in the texture of lard or butter.

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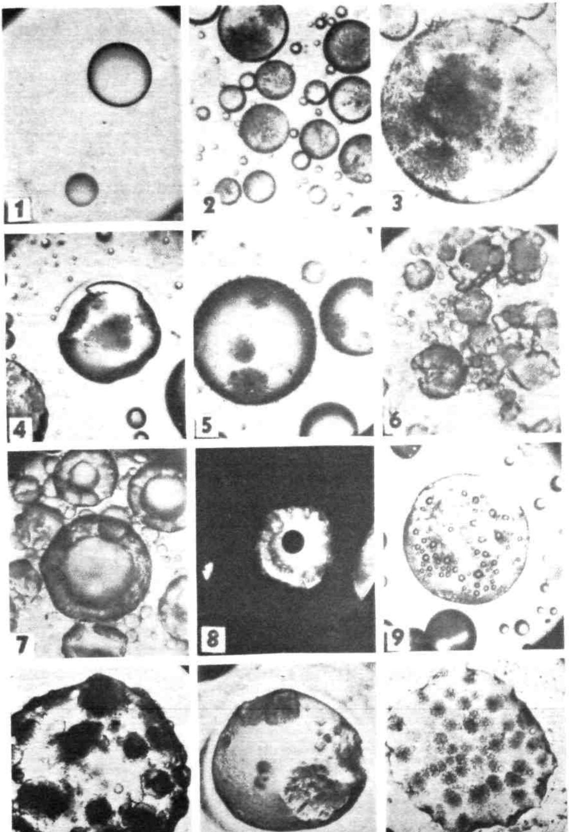
Plate I. Figs. 1 – 12. *Globules of butterfat. All figures ×80.* Fig. 1. *Submerged globules before the formation of any crystalline material.* Fig. 2. *Typical globules of normal butterfat after 2 or 3 days' incubation at 25° C.* Fig. 3. *Crystal formation in large surface globule. A very small amount of sodium chloride had been added to the agar in which this globule was suspended, to enlarge the size of the globules and hasten crystallization.* Fig. 4. *Addition of 5% sodium chloride. After 3 days' incubation at 25° C. Note coarse crystal masses and shrinkage of the globule in the agar mould.* Figs. 5 and 6. *Addition of 5% sodium chloride. After 20 hours' and 8 days' incubation respectively. Note appearance of disintegration.* Fig. 7. *Globules 28 days old dispersed in beef extract agar. These typical "crater" forms are either on or near the surface. They frequently form in a much shorter period.* Fig. 8. *Similar to Figs. 5 and 6, except that india ink has been flooded over the plate. This demonstrates the spherical bodies within the oily centre of these crater forms. Frequently the india ink very quickly adheres to the surface of these spherical bodies.* Fig. 9. *Droplets in a surface globule of partially oxidized butter. Note similar droplets in Fig. 12.* Fig. 10. *pH about 4.4. Agar not jellied. Globules float at surface.* Fig. 11. *pH about 6.0.* Fig. 12. *pH about 8.8. A surface globule. Note shrinkage of the globule, finer crystal masses and droplets.*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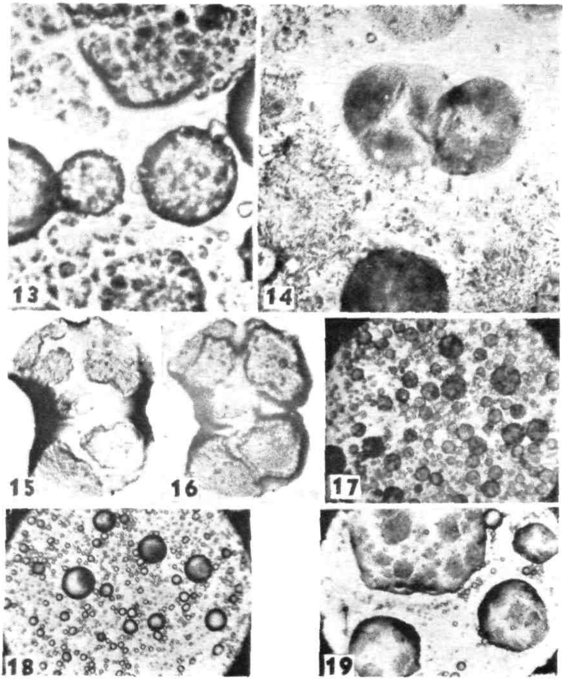

PLATE II, FIGS 13 – 19. FIG. 13. *Globules made from old rancid butter, at the end of 24 hr. Crystal formation was very rapid.* ×100. FIG. 14. *Butterfat added to 20% brine solution and shaken vigorously for 20 min. Fat washed free of salt and dispersed in agar. Greatly enlarged, showing the variation of crystal bodies in a portion of a surface globule.* ×440. FIGS. 15 AND 16. *The same globule of normal butterfat at different temperatures. Decrease in temperature from 25° C. to below 15° C. caused the formation of the wax-like edges around the crystal masses.* ×100. FIG. 17. *This plate was placed in the ice box for 1 hr. immediately after being poured, and then incubated for 8 days at 25° C. The initial rapid crystallization resulted in finer crystals and more uniform globules.* ×32. FIG. 18. *Normal butter fat globules at 24 hr.* ×32. FIG. 19. *The same to which 5% peptone had been added.* ×32

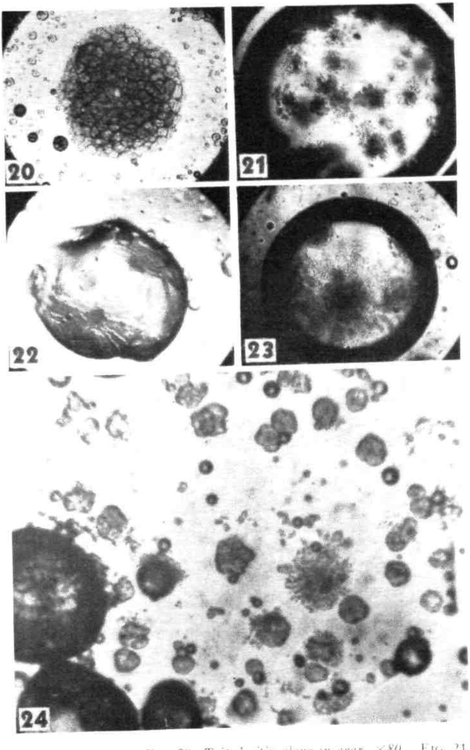

PLATE III, FIGS 20 – 24. FIG. 20. *Tripalmitin alone in agar.* ×*80.* FIG. 21. *A large surface globule of a mixture of tristearin and triolein.* ×*160.* FIG. 22. *Surface and submerged globules of a mixture of tripalmitin and triolein.* ×*160.* FIG. 23. *Globules of mixtures of tristearin, tripalmitin, and triolein.* ×*160.* FIG. 24. *Surface and submerged globules of a mixture of tristearin and triolein. In the large surface globule there are both the commonly illustrated compact crystal masses and the feathery type more frequently met with in butterfat globules.* ×*440.*

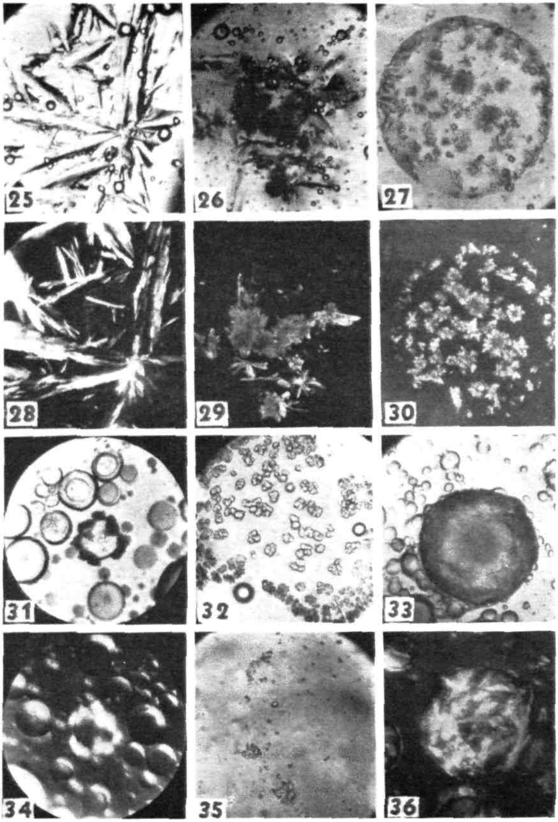

PLATE IV. FIGS. 25 – 36. *Crystals of pure fats and fatty acids in globules of triolein photographed with and without polaroid discs.* FIGS. 25 AND 28. *Trilaurin and triolein.* ×*160.* FIGS. 26 AND 29. *Trilaurin and triolein.* ×*80.* FIGS. 27 AND 30. *Trimyristin and triolein.* ×*80.* FIGS. 31 AND 34. *Myristic acid and triolein.* ×*80.* FIG. 32. *Stearic acid and triolein.* ×*160.* FIGS. 33 AND 36. *Stearic, palmitic, myristic, and lauric acids in globules of triolein.* ×*80.* FIG. 35. *Large surface globules of myristic acid and triolein.* ×*80.*

VARIETAL DIFFERENCES IN BARLEYS AND MALTS

IX. CARBOHYDRATE FRACTIONS OF BARLEY AND THEIR CORRELATIONS WITH TOTAL NITROGEN AND 1000-KERNEL WEIGHT[1]

By C. A. Ayre[2], H. R. Sallans[3], and J. A. Anderson[4]

Abstract

Samples of 12 varieties of barley from 12 experimental stations in Canada were analysed for starch, barley extract, and crude cellulose plus lignin. Nine six-rowed varieties averaged 3.4% lower in starch, 3.7% lower in extract, and 1.4% higher in cellulose plus lignin than three two-rowed varieties. Maximum and minimum values of the means for the six-rowed varieties were: starch, 55.0% and 52.4%; extract, 77.2% and 74.3%; and crude cellulose plus lignin, 11.3% and 10.1%. Environment had a significant effect. Maximum and minimum values for station means were: starch, 59.2% and 51.7%; extract, 80.8% and 73.6%; crude cellulose plus lignin, 11.0% and 9.6%.

Correlation coefficients of carbohydrate fractions with total nitrogen were not significant between varieties but highly significant within varieties for starch, $r = -0.95$, and extract, $r = -0.91$. Partial coefficients, independent of nitrogen between starch and 1000-kernel weight, were significant: between varieties, 0.62; within varieties, 0.75. Those with crude celluiose plus lignin did not attain significance.

Starch and barley extract are closely associated within varieties, $r = 0.961$, and between varieties, $r = 0.982$. The insoluble cellulose-lignin fraction gave negative inter-varietal associations with starch, $r = -0.952$, and barley extract, $r = -0.968$. Similar relations within varieties are obscured in the simple coefficients but the corresponding partials independent of total nitrogen are significant.

Introduction

As progress was made in the investigation of barley and malt properties and of the relations among them, it appeared that the possibilities for predicting malt extract from the results of barley analyses might repay further study. It therefore seemed desirable to make provision for a more adequate investigation of this subject by adding the results of determinations of various carbohydrate fractions to the data already collected on other properties of Canadian barleys.

Since starch makes the greatest contribution to the extract yield of malt, a determination of it was essential. Of the various methods available, that of Lintner-Schwartz (6), which involves dispersion in sulphuric acid and subsequent polarization, was selected as the most suitable for the present study. It is doubtful whether the method determines starch with accuracy, but it is

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comparatively rapid, yields reproducible results and thus has several advantages for large scale routine investigations. In the present instance it is more important to know whether the Lintner-Schwartz method yields results that are valuable for prediction purposes than to know if it determines starch alone and is not affected by other minor constituents of the barley.

It also seemed advisable to determine total barley extract. Most previous investigators have attempted to devise methods that will give identical values for barley extract and the extract of the malt made from it. Owing to the loss of potentially extractable barley matter during malting, this does not seem to be logical since, to attain these conditions, barley extract must be under-estimated. If a constant relation can be shown to exist between barley and malt extracts, it is of no consequence whether the values are identical since prediction can be made by calculation. In these circumstances a method that would determine all the potentially extractable material of the barley was sought for the present investigation.

To realize these conditions it was essential to grind the barley finely, and a ball mill was used for this purpose. After some experimental work, a modification of the Lüers-Miller (5) method was applied to the ground barley. It involves mashing with the addition of preparations of liquefying and saccharifying enzymes and a subsequent determination of the specific gravity of the resulting wort. To obtain additional information, the total nitrogen of the wort was determined. It was thus possible to calculate the non-protein extract yield of the barley and this could be compared with starch content.

Since Bishop's work (3, 4) has shown that malt extract is inversely correlated with the insoluble carbohydrates of the barley, it also seemed desirable to determine this fraction. Bishop's method, treatment with boiling 0.5 *N* sulphuric acid followed by boiling 0.5 *N* sodium hydroxide, determines an anomalous fraction and for this reason is not particularly attractive. A more specific fraction can be obtained by the method of Waksman-Stevens (7), which determines roughly the combined cellulose plus lignin. This method is no less empirical, but it appeared that it might yield additional information that could not be obtained merely by repeating Bishop's work.

In the present study, 144 samples of barley were analysed for starch, extract, extract nitrogen, and the acid resistant carbohydrate residue which can be called crude cellulose plus lignin. A statistical study of the relations among these constituents and total nitrogen and 1000-kernel weight was also undertaken. The data and statistics are more or less self-explanatory and discussion of them has therefore been reduced to a minimum.

Materials and Methods

Materials

The barley samples used in the present investigation were described in detail in Part I of this series of papers (1). Briefly, they consisted of 144 samples representing 12 varieties grown at each of 12 widely separated experimental stations in Canada. The varieties are listed in Table I and the stations in Table II. For the purpose of the determinations described in the

present paper, the samples were ground first in a Wiley mill and subsequently for 24 hr. in a ball mill. To provide a check on the precision of analyses, duplicate determinations were made on one-third of the samples. These were selected at random after imposing the limitation that four samples of each variety and four samples from each station should be chosen.

Starch

A 1.25-gm. sample of barley flour was weighed into a 100-ml. glass centrifuge tube. It was then worked into a smooth paste with 2 ml. of 95% alcohol, and 25 ml. of sulphuric acid (sp. gr. 1.4) was added with vigorous stirring to prevent the formation of lumps. The tube was placed in a water bath at 20° C. and the mixture stirred at intervals of five minutes. At the end of one hour, 20 ml. of sulphuric acid (sp. gr. 1.3) and 5 ml. of a 2% solution of phosphotungstic acid were added with vigorous stirring. The tube was then centrifuged and the slightly cloudy supernatant liquid was decanted through a filter paper (Whatman No. 44). Six polarimetric readings were made on the clear filtrate. The average reading was converted to per cent starch by multiplying by the factor 10.1, and the resulting figure corrected for the moisture content of the original sample. The standard error of a single determination was 0.25% starch, or 0.5% of the mean value, 54.3%.

Barley Extract

A 15-gm. sample of barley contained in a brass mashing beaker was made into a smooth paste with 50 ml. of distilled water, and 25 ml. of an 0.8% solution of "rapidase"* was added. The beaker was held at 20° C. overnight in a controlled water bath. In the morning, the thin mash was boiled on a hot plate for 10 min., with constant stirring. After cooling in a mash bath to 45° C., 25 ml. of a 1.2% solution of malt diastase and sufficient distilled water to cover the stirrer were added. The temperature was then raised to 48–50° C., held there for 10 min., and then increased at a rate of one degree per minute to 75° C., where it was held for 30 min. The mixture was cooled to room temperature, made up to a weight of 135 gm., stirred and transferred to a dry centrifuge bottle. After centrifuging, the supernatant liquid was decanted through a folded filter paper and the specific gravity of the filtrate was determined. Blank determinations were made on the enzyme solutions and, after correcting for this blank, barley extract was calculated in the usual manner. The standard error of a single determination proved to be 0.23% in barley extract, or 0.3% of the mean value, 76.0%.

The nitrogen in 25 ml. of the barley extract was determined by the Kjeldahl method and is reported as a percentage of the original sample of barley.

Crude Cellulose Plus Lignin

The small amount of material remaining in the mash beaker was washed into the centrifuge bottle, which already contained the bulk of the residue from the barley extract determination. The material was mixed with a

* *The rapidase and malt diastase preparations used in this investigation were supplied through the courtesy of the Wallerstein Laboratories, 180 Madison Ave., New York City, U.S.A.*

small quantity of water, centrifuged, and the supernatant washings decanted. The residue was mixed with 100 ml. of a 2% solution of hydrochloric acid and boiled under a reflux condenser for five hours. For this purpose, the centrifuge bottle was immersed in an oil bath maintained at 117° C. The mixture was then cooled and centrifuged. After decanting the clear supernatant liquid, the solid residue was transferred to a Buchner funnel containing a tared filter paper (Whatman No. 41) and washed six successive times with boiling water. The acid-insoluble material and filter paper were dried for five hours in an air oven at 100° C. and then weighed. The nitrogen content of the residue was determined by the Kjeldahl method, and non-protein acid-resistant residue was calculated after converting nitrogen to protein with the factor 6.0. The non-protein acid-resistant fraction is reported as crude cellulose plus lignin. The standard error of a single observation was 0.13%, or 1.3% of the mean value, 10.4%.

Results and Discussion

Varietal Differences

The results of the investigation are summarized in Table I as mean values for each variety over all 12 stations. Owing to the differential effect of environment on varieties, these did not fall in the same order at each station. Analyses of variance were necessary to determine whether the differences between the means for individual varieties could be considered significant. The results of these analyses are given in Table III but are summarized in the last line of Table I as necessary differences between means required for a 5% level of significance, i.e., for odds of 19 to 1 that real differences exist between varieties.

TABLE I

MEAN VALUES FOR EACH VARIETY, FOR STARCH, CRUDE CELLULOSE-LIGNIN, AND BARLEY EXTRACT

Class	Variety	Starch, %	Crude cellulose-lignin, %	Extract, %	Extract nitrogen, %
Six-rowed rough-awned	O.A.C. 21	53.6	10.5	75.3	0.64
	Mensury, Ott. 60	53.5	10.3	75.2	0.65
	Olli	54.7	10.1	77.2	0.71
	Peatland	54.3	10.2	75.9	0.69
	Pontiac	53.0	11.1	74.6	0.63
Six-rowed smooth-awned	Nobarb	55.0	10.2	76.5	0.56
	Regal	52.7	10.9	74.3	0.62
	Velvet	52.4	11.0	74.5	0.62
	Wisconsin 38	53.2	11.3	74.4	0.58
Two-rowed rough-awned	Charlottetown 80	56.9	9.4	78.5	0.66
	Hannchen	57.3	9.2	79.5	0.67
	Victory	56.8	8.9	79.0	0.64
Mean, over all varieties		54.5	10.3	76.2	0.64
Maximum spread		4.9	2.4	5.2	0.15
Necessary difference, 5% level		0.66	0.32	0.64	0.013

A comparison of the actual differences between varietal means, with the necessary differences required for a 5% level of significance, shows clearly that there are real differences between varieties for all properties measured. This was to be expected since varietal differences have been shown for all properties reported in the present series of papers.

The data show clear-cut differences between two-rowed and six-rowed varieties. While the former are higher in starch and barley extract, they are lower in crude cellulose plus lignin. This is in agreement with the findings of Bishop (3), who attributes the higher yield of malt extract obtained from two-rowed varieties to this fact. Among the six-rowed varieties there appears to be some indication that the rough-awned types are higher in starch and extract content than the smooth-awned types. However, in view of the comparatively small number of varieties under study and the anomalous behaviour of Nobarb, it is impossible to state definitely that differences exist between rough-awned and smooth-awned barleys with respect to their carbohydrate composition.

There is some evidence that the smooth-awned varieties tend to be lower in extract nitrogen. This is in agreement with the evidence presented in Part I (1) and Part V (2) that these barleys tend to be low in salt-soluble nitrogen and that the malts from them tend to be low in wort nitrogen.

Station Differences

The analytical results are summarized in Table II as means, over all varieties, for each station. The stations are listed in order of increasing nitrogen content of the barleys in order to facilitate examination of the relations between nitrogen and other constituents, a subject which is discussed in the next section.

TABLE II

MEAN VALUES FOR EACH STATION, FOR NITROGEN, STARCH, CRUDE CELLULOSE-LIGNIN, AND BARLEY EXTRACT

Station	Nitrogen, %	Starch, %	Crude cellulose-lignin, %	Extract, %	Extract nitrogen, %
Nappan	1.54	59.2	10.0	80.8	0.52
Fredericton	1.74	57.7	10.8	78.2	0.54
Ste. Anne de Bellevue	1.93	56.1	11.0	77.5	0.56
Ste. Anne de la Pocatière	2.28	54.2	10.9	76.8	0.62
Lethbridge	2.29	55.1	9.6	77.9	0.70
Winnipeg	2.33	53.6	10.2	74.7	0.64
Brandon	2.36	54.8	9.8	76.5	0.64
Guelph	2.38	52.4	10.6	74.6	0.68
Ottawa	2.53	53.3	9.9	75.5	0.68
Lacombe	2.67	52.7	10.0	74.4	0.70
Beaverlodge	2.67	51.7	10.4	73.6	0.67
Gilbert Plains	2.69	52.7	9.9	74.2	0.72
Mean, over all stations	2.28	54.5	10.3	76.2	0.64
Maximum spread	1.15	7.5	1.4	7.2	0.20
Necessary difference, 5% level	0.079	0.66	0.32	0.64	0.013

The data show that environmental differences resulted in the production of a series of samples showing fairly wide variations with respect to all properties measured. Moreover, comparison of the spreads between station means with the necessary difference required for a 5% level of significance, shows conclusively *that environment has a significant effect on each of the various constituents. It is interesting to note* that the maximum spreads between station means for starch and barley extract are somewhat greater than the corresponding spreads between varietal means (see Table I), whereas for the cellulose-lignin fraction the spread between varietal means is considerably greater than that between station means, i.e., 2.4% as against 1.4%. Since the cellulose and lignin in the barley kernels exist mainly in the hull, the data suggest that variety has a greater effect than environment on the total quantity and thickness of the hull. This assumption seems justified, since the data on which it is based were obtained by analyses of samples representing a very wide range of both varieties and environments.

TABLE III

ANALYSIS OF VARIANCE FOR BARLEY PROPERTIES: MEAN SQUARES

Barley property as % dry matter	*Variance due to*		
	Stations	Varieties	Interaction
Starch	61.474**	35.278**	0.649
Crude cellulose-lignin	2.563**	7.079**	0.151
Extract	54.650**	43.348**	0.612
Extract nitrogen	0.05531**	0.02105**	0.00108
Degrees of freedom	11	11	121

Correlations with Total Nitrogen

The correlations between total nitrogen and other constituents were studied by calculating the correlation coefficients for varietal and station means. The resulting statistics are given in Table IV.

TABLE IV

INTER-VARIETAL AND INTER-STATION CORRELATION COEFFICIENTS BETWEEN NITROGEN CONTENT AND BARLEY PROPERTIES

Barley property as % dry matter	Correlation coefficient	
	Inter-varietal	Inter-station
Starch	-.399	-.953**
Crude cellulose-lignin	.301	-.334
Extract	-.401	-.908**
Extract nitrogen	.241	.924**

Since none of the inter-varietal correlation coefficients are significant, the investigation yields no evidence of inter-varietal relations between total nitrogen and any of the other properties listed. On the other hand, several of the inter-station correlation coefficients are highly significant. With respect to station means, total nitrogen is significantly and negatively correlated with starch and extract and significantly and positively correlated with the nitrogen in the barley extract. Thus environmental conditions, which tend to increase total nitrogen, tend also to increase barley extract nitrogen, and to decrease starch and barley extract. These intra-varietal relations were certainly to be expected and no further discussion of them seems necessary.

Correlations with 1000-kernel Weight

The inter-varietal and inter-station correlation coefficients between 1000-kernel weight and other properties are given in the first two columns in Table V. Owing to the association between 1000-kernel weight and total nitrogen, it appeared that these statistics might reflect correlations with nitrogen, rather than actual correlations with 1000-kernel weight. The possible effects of nitrogen were therefore eliminated by calculating the partial correlation coefficients for 1000-kernel weight and each property, independent of total nitrogen. These correlation coefficients are listed in the last two columns of Table V. In general, the simple and partial coefficients are of the same order of magnitude, which indicates that nitrogen content has little or no influence on the relations.

TABLE V

INTER-VARIETAL AND INTER-STATION CORRELATION COEFFICIENTS BETWEEN 1000-KERNEL WEIGHT AND BARLEY PROPERTIES, AND PARTIAL CORRELATION COEFFICIENTS INDEPENDENT OF NITROGEN CONTENT

Barley property as % dry matter	Correlation coefficient		Partial correlation coefficient	
	Inter-varietal	Inter-station	Inter-varietal	Inter-station
Starch	.690*	.688*	.615*	.746**
Crude cellulose-lignin	−.621*	−.054	−.572	.149
Extract	.639*	.688*	.547	.605*
Extract nitrogen	−.300	−.552	−.206	−.221

Starch content and barley extract are positively correlated with 1000-kernel weight both within and between varieties. This is in agreement with the common assumption that barleys having larger kernels should yield a greater proportion of extractives due to a lower husk to endosperm ratio. Since the major portion of the cellulose and lignin occurs in the hulls of the barley, a negative association would be expected between this fraction and 1000-kernel weight. The exceptionally low value of the inter-station coefficient may be due to variations in the ash content of the residue from barley grown on different soil types. From the comparatively low values of all the correlation coefficients, it is apparent that 1000-kernel weight is a very poor indication of the potential extractives in a barley, and its importance in evaluating barley for malting purposes appears to have been over-emphasized.

No association exists between nitrogen content of the barley extract and 1000-kernel weight. This probably explains in part the lower degree of association between barley extract and kernel weight than between starch and kernel weight.

Correlations among the Carbohydrate Fractions

Correlation coefficients showing the interrelations of the various barley fractions under study are listed in Table VI. The first four columns contain the simple coefficients and the partial coefficients, independent of total nitrogen, for the inter-varietal correlations. The last four columns contain the corresponding data for inter-station correlations.

TABLE VI

CORRELATION COEFFICIENTS (SIMPLE AND PARTIAL, INDEPENDENT OF TOTAL NITROGEN) AMONG STARCH, EXTRACT, CELLULOSE-LIGNIN RESIDUE, AND EXTRACT NITROGEN OF BARLEY

Type of correlation	Independent variable	Inter-varietal coefficients			Inter-station coefficients		
		Cellulose-lignin	Barley extract	Extract nitrogen	Cellulose-lignin	Barley extract	Extract nitrogen
Simple	Starch	−.952**	.982**	.312	.028	.961**	−.859**
	Cellulose-lignin		−.968**	−.433		.027	−.498
	Barley extract			.074			−.749**
Partial, independent of total nitrogen	Starch	−.953**	.979**	.458	−.969**	.752**	.190
	Cellulose-lignin		−.971**	−.543		−.665*	−.557
	Barley extract			.355			.439

A very close positive association is shown to exist between starch content and barley extract both within and between varieties. This was to be expected since starch is the major constituent contributing to extract yield. But in addition to starch, other carbohydrates also contribute to extract and it appears that these other carbohydrate fractions must also be related to starch content. If barley extract is corrected for its nitrogen content the simple coefficients between starch and non-protein extract are 0.98 and 0.97 for inter-varietal and inter-station correlations. While these coefficients are slightly higher than those for total extract, it is evident that the extract nitrogen has an almost negligible influence on the starch-extract relations. These considerations suggest that in addition to a carbohydrate regularity within samples of the same variety (4), similar regularities exist for samples of different varieties grown under the same environmental conditions.

As would be expected, a highly significant negative association is indicated between the insoluble cellulose-lignin fraction and both starch and barley extract. These relations are perfectly clear-cut in the simple inter-varietal coefficients. They are, however, masked by common associations with total nitrogen in the inter-station simple coefficients but are readily proved to exist by calculating the partial coefficients independent of the effects of total nitrogen.

Within varieties, no relation appears to exist between the nitrogen content of the barley extract and the carbohydrate fractions. The simple interstation coefficients obtained between this property and both starch and barley extract are significant, but the partial coefficients independent of total nitrogen show clearly that these effects are due to common association of the carbohydrates and extract nitrogen with total nitrogen (cf. Table IV).

The most interesting feature of these relations is a regularity in the carbohydrate composition of barley (4) which appears to hold both within and between varieties. This is contrasted with a regularity in the protein composition which holds only within varieties (1). The partial correlation coefficients (cf. Table VI) and simple coefficients (cf. Table IV) indicate that within varieties there is an interdependence of the carbohydrate regularities on the nitrogen regularities, whereas between varieties no relations appear to exist between the nitrogen and carbohydrate contents of the barleys.

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THE USE OF WATER BY WHEAT PLANTS WHEN INOCULATED WITH *HELMINTHOSPORIUM SATIVUM*[1]

BY B. J. SALLANS[2]

Abstract

The transpirational histories of wheat plants, inoculated with *Helminthosporium sativum* Pamm., King & Bakke to produce root rot, showed a marked reduction in water loss during the early stages of growth, when compared with uninoculated plants. This was accompanied by a corresponding reduction in the transpiring areas owing to reduced lengths and widths in the second, third, and fourth leaves to appear. As the plants entered into the early stages of elongation of the culm the transpirational story reflected a recovery in the inoculated plants. The later leaves to appear were longer with greater transpiring areas than in uninoculated plants. This fact combined with the death of the first three or four leaves resulted in greater transpirational and photosynthetic areas in the inoculated plants with consequent increased yields of dry matter. Probably similar recovery does not occur under competitive field conditions, where weeds and healthy wheat plants are present. Poor light conditions and low soil moistures were not conducive to recovery of inoculated plants. The water requirements of wheat did not appear to be affected significantly by inoculation. Of two varieties of spring wheat, Reward was more severely injured initially and recovered more rapidly than Marquis. Several possible explanations of the recovery recorded here are discussed.

Introduction

A disease of wheat-stem bases and roots caused by *Helminthosporium sativum* Pamm., King & Bakke is of widespread occurrence in Western Canada. It is one of several diseases affecting the underground portions of the stems, the crown, the subcrown internodes, and roots, which have similar symptoms and which collectively are known as "common root rot" or "foot rot". This disease has several manifestations such as pre-emergence blight, seedling blight, tiller blight, and prematurity blight. However, by far the most important phase of the disease under conditions in Western Canada is one in which the underground parts of the plants become lesioned with external and internal discolorations of the tissues, the plants being more or less stunted and their yields of grain reduced; but a phase in which the plants are not usually killed outright.

The injury to the plants and the reduction in yield of grain are difficult to estimate under these conditions. The diseased plants seldom occur in definite patches, but rather as individuals scattered among healthy or slightly diseased plants. Thus, it is not possible, ordinarily, to compare yields of affected areas of a field with yields of unaffected parts, as may be done with certain other root rots of cereals such as take-all (*Ophiobolus graminis* Sacc.) of wheat. While the author (8) has found that individual plant yields may be reduced

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by 30 to 45% coincident with the presence of severe subcrown internode lesions, as compared with unlesioned or slightly lesioned neighbouring plants, the problem of competition for soil moisture and nutrients complicates the determination of actual net losses in yield per unit area of land.

Under the prevailing climatic conditions of Western Canada, soil moisture may be considered the most important limiting factor in wheat production. Any economy or wastage of soil moisture, as influenced by the practice of summerfallow or the growth of weeds, generally has an important effect upon the following cereal crops. The influence of *H. sativum* upon the transpiration of water by wheat plants when the underground parts are attacked was considered a necessary study in an evaluation of the disease.

It is well known that certain foliage diseases decrease or accelerate the transpiration of water by plants. A number of papers, of which Bever's (1) is a recent example, show that cereal rusts in particular increase water loss. The influence of parasites of underground portions of the plant, however, seems to have received little attention. Burkholder (2) reported transpiration studies on bean plants infected by the root parasite, *Fusarium Martii* f. *Phaseoli* Burkh. In the uninoculated series the water requirement increased with increased soil moistures. There appeared to be reduced water requirements in the inoculated plants, especiallly at the lower soil moistures. The transpirational data indicate very much reduced use of water by the inoculated plants, this being most evident in dry soil and less so in medium and wet soil. Linford (4) studied the transpirational history of peas infected by the *Fusarium* wilt organism. He found a decreasing amount of transpiration in diseased plants up to the time when wilting became evident. Most plants then showed a slightly increasing rate followed in a few days by a rapid decrease when wilting was complete.

In the present work data were taken in such a way as to secure transpirational histories of plants inoculated with *H. sativum* as compared with uninoculated plants, in addition to information on water requirements.

Since considerable differences were observed in the amounts of water transpired by plants owing to the presence or absence of root rot, it was thought that a study of transpiring areas might yield interesting information. Considerable stunting was observable in the seedling stage of inoculated plants. Yet under the conditions of some of the experiments there was an evident recovery in size, further indications of which were given by the data on water transpired per plant. Leaf areas were measured in Experiments III and IV.

The results reported in this paper were obtained under the artificial conditions of the greenhouse in winter and are not strictly applicable in the interpretation of water relations in the field.

Methods

The gravimetric method was adopted to measure transpirational losses. Regular weighings were made at intervals of two days to secure consecutive data from the early seedling stage to maturity, and losses were made up by

adding water from a burette. The pots or crocks were regularly and systematically interchanged on the greenhouse bench to compensate for environmental differences.

Of the various methods available for measuring transpiring areas, the one selected involved direct measurements and calculation. The leaves could thus be measured at any time during their functional period without removal from the plants. Leaf blade areas were calculated from the leaf length measured to the nearest millimetre, the greatest width estimated to the nearest tenth of a millimetre, and a factor. The factor was determined by measuring the widths of the leaf at one-centimetre intervals along its length. The mean width was then multiplied by two and divided by the maximum width to give the desired factor. The factor obtained and its standard deviation were 1.611 ± 0.013. Inoculation, soil moistures, and varietal differences did not significantly affect the factor. The first foliage leaf differs in shape from succeeding ones and 1.84 was the factor determined for it. In addition to the areas of leaf blades the exposed areas of culms and leaf sheaths were estimated.

Some sets of data were examined statistically by Fisher's analysis of variance method as outlined by Goulden (3).

Experiment I

A preliminary experiment was started in the greenhouse at Saskatoon, Saskatchewan, on February 17, 1936. Sixteen crocks of one Imperial gallon were used. The soil was not sterilized. Four crocks were seeded to Marquis and four to Reward wheat as controls. Similarly, four crocks were seeded to each variety, with seed that had been inoculated with conidia and mycelium of *H. sativum*. In addition, five crocks were prepared in the same manner but left unseeded. Water was added to bring the soil moisture to 50% of the moisture-holding capacity of the soil. The amount of water lost by transpiration was estimated by subtracting the average amount of water lost by evaporation from the five unplanted crocks from the total amount lost from each planted crock.

On Feb. 27 the plants that emerged were thinned to a uniform stand of 36 per crock. This was done by first removing excessively stunted and blighted seedlings and then removing at random the seedlings in excess of 36. On March 13 a further reduction to 12 per crock was made, again at random. The 24 plants removed from each crock were used to determine dry weight per plant. By considering the removed seedlings as samples of the whole population of 36 plants, estimates of the water requirements of the seedlings were made. These data are given in Table I and the analyses of variance in Table II.

While the dry weights per plant were significantly reduced by inoculation with *H. sativum*, there were corresponding reductions in the amounts of water transpired, hence no significant differences in water requirements due to inoculation were obtained. The interaction IU × Variety is significant for

TABLE I

DATA AT THE SEEDLING STAGE IN EXPERIMENT I

Variety	Treatment	Dry weight of plants, gm.	Water transpired, gm.	Water requirement, gm.
Marquis	Uninoculated	2.21	1162	350
Marquis	Inoculated	2.00	1017	346
Reward	Uninoculated	2.30	1190	344
Reward	Inoculated	1.73	783	323

TABLE II

ANALYSES OF VARIANCE OF DATA SUMMARIZED IN TABLE I

Source of variance	D.f.	Mean variances		
		Dry weight of plants	Water transpired	Water requirements
Replicates	3	0.0381**	11975	350.7
Treatments	3	0.2602**	138968**	581.6
IU	1	0.6123**	305533**	637.6
Variety	1	0.0333*	42333*	826.6
IU × Variety	1	0.1350**	69037**	280.6
Error	9	0.0043	5251	193.6

*Throughout this paper, "IU" will be used in analyses of variance tables for "inoculated" and "uninoculated". The 5% and 1% levels of significance will be designated by * and ** respectively.*

dry weights of plants and water transpired. It appears that Reward was more susceptible than Marquis to injury from the fungus in this experiment.

The experiment was continued to maturity of the plants, when yields and disease ratings were obtained. Table III gives in condensed form the transpirational histories of plants in the various treatments. The water losses are given for 11 periods of 9 and 10 days each.

The relation of the amount of water used by the inoculated plants to that used by the uninoculated plants is graphically represented in Fig. 1. The

TABLE III

THE WATER LOSS BY TRANSPIRATION IN GRAMS PER PLANT IN EXPERIMENT I

Variety	Treatment	Periods										
		1	2	3	4	5	6	7	8	9	10	11
Marquis	Uninoculated	14	35	53	70	96	103	120	118	95	101	24
Marquis	Inoculated	11	34	53	77	106	110	126	122	96	103	25
Reward	Uninoculated	14	35	58	82	111	105	111	96	42	10	0
Reward	Inoculated	8	29	49	77	116	115	122	108	58	18	1

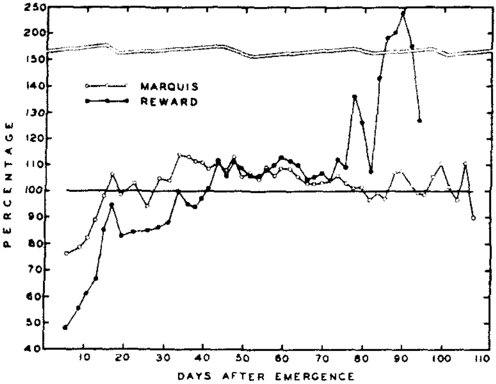

FIG. 1. *Graphs representing transpirational histories in Experiment I of Marquis and Reward wheats inoculated with Helminthosporium sativum, expressed as percentages of their respective uninoculated controls. The horizontal straight line at the 100% mark represents the controls. The data were recorded to maturity of the control plants.*

figure was prepared from data for periods of 2 or 4 days in the seedling stage and of 2 days thereafter. In Table III these data were condensed to fewer periods. The total estimated transpiration, for the four replicates of each variety inoculated with *H. sativum*, is expressed as a percentage of the corresponding figure for uninoculated plants. The initial low transpiration by inoculated plants, especially in Reward, was followed by a period of recovery, following which the inoculated plants used more water than the controls. The Reward control matured about four days before the inoculated plants, hence the greatly increased ratio in use of water by the latter toward the end of the experimental period.

Yields of grain, total dry weight of tops, and the total amount of water used per plant are given in Table IV. Water requirements in grams for each gram of oven-dry matter were calculated. Table V gives the analyses of variance of these data.

The yield of grain, the total dry plant weights, and the total water used by inoculated plants were significantly higher than in the uninoculated plants. The inoculated plants appeared to be significantly more economical in the use of water than the controls. The non-significance of the interaction Variety × IU in any of the sets of data indicates that Marquis and Reward responded similarly to inoculation.

The analysis of the average weight per seed data shows no difference ascribable to variety or inoculation with *H. sativum*. There are, however, significantly influenced numbers of seeds per plant due to treatments. The

TABLE IV

YIELDS OF GRAIN, DRY WEIGHT OF PLANT TOPS, WATER USED PER PLANT, AND WATER REQUIREMENTS IN EXPERIMENT I

Variety	Treatment	Yield of grain		Weight per seed, mg.	Dry weight of plant tops, gm.	Total water used per plant, gm.	Water requirements, gm.
		Weight, gm.	Seeds, no.				
Marquis	Uninoculated	9.1	261	35.3	27.5	829	361
Marquis	Inoculated	9.8	283	34.7	30.2	863	343
Reward	Uninoculated	9.4	273	34.5	25.3	664	315
Reward	Inoculated	10.9	321	34.0	28.3	701	297

TABLE V

ANALYSES OF VARIANCE OF THE DATA SUMMARIZED IN TABLE IV GIVING THE MEAN VARIANCES

Source of variance	D.f.	Mean variances					
		Weight of grain	No. of seeds	Weight per seed	Dry weight of plant tops	Total water used per plant	Water requirement
Replicates	3	0.11	17	2.32	0.92	532	21.7
Treatments	3	2.48**	2683**	1.22	16.72**	37199**	3269.0**
Variety	1	1.96**	2500**	2.40	16.40**	106439**	8418.0**
IU	1	4.84**	4900**	1.21	33.64**	5148**	1387.5**
Variety × IU	1	0.64	650	0.04	0.12	11	1.5
Error	9	0.14	169	0.83	0.57	422	121.1

increased yields in the inoculated plants are not due, therefore, to kernels of different weight, but to the numbers of kernels produced by each plant. Thus, it appears that any influence exerted by inoculation with *H. sativum* on yield occurred before seed was set and not during the period of filling of the grain.

Experiment II

The second experiment was started in the greenhouse at the University of Wisconsin on November 18, 1936. No artificial light was supplied, consequently the plants grew poorly during the period of short days. Reward finally headed, but Marquis remained in a vegetative condition until the experiment was terminated on March 20. Under these conditions there was no marked tendency for the inoculated plants to recover, indicated in the transpirational data. Since the results of this experiment are in entire agreement with those of Experiment IV, in so far as they may be compared, the data need not be given here.

Experiment III

The third experiment, conducted at the University of Wisconsin, was started February 13, 1936, in a 20° C. greenhouse. Four levels of soil moisture were used, namely 30, 45, 60, and 75% of the M.H.C. of the soil. One variety of wheat, Reward, was sown, the seed for one-half the containers at each moisture level being inoculated with *H. sativum*. As the experiment was conducted in quadruplicate there were 32 containers.

The data on transpiration were assembled into nine periods. The first eight periods consist of ten days each, and the ninth period of six days. The plants were for the most part fairly well matured when harvested on May 26. The data are given in Table VI.

TABLE VI

TRANSPIRATIONAL DATA FOR EXPERIMENT III

M.H.C. of soil, %	Treatment	Periods								
		1	2	3	4	5	6	7	8	9
75	Uninoculated	642	1585	2423	2899	3237	2108	2491	1124	110
75	Inoculated	472	1087	2032	3058	3832	2723	3296	1949	559
60	Uninoculated	619	1354	2045	2621	3157	2486	3083	1955	445
60	Inoculated	415	989	1948	2814	3566	2881	3652	2634	820
45	Uninoculated	391	870	1022	1130	1467	1361	1817	1419	562
45	Inoculated	227	587	907	835	999	920	1305	1147	506
30	Uninoculated	73	282	508	586	636	523	665	498	160
30	Inoculated	74	180	368	504	476	397	492	401	163

The water transpired in the early periods is small compared to that transpired by the full-grown plants, and a pooled estimate of variance cannot be used in evaluating main effects and interactions involving periods. To overcome this difficulty the data were transformed to logarithms to minimize the contrasts between periods. Williams (11) has made a similar use of logarithms in analysing entomological data.

To test whether the error variances were markedly different from period to period in the transformed data, use was made of the method of Yates and Cochran (12) in determining whether error mean variances of the same experiment at different stations and in different years were in fact the same. The error variances for the periods were sufficiently alike to be considered the same. Table VII gives the mean logarithms of the data summarized in Table VI, and Table VIII the analysis of variance of the transformed data.

The mean variances for periods and moistures are highly significant. The amount of water transpired by plants grown at the higher soil moistures is greater than at lower soil moistures due to the larger leaf areas in part and to the greater ease with which water may be withdrawn from the soil. The mean variance for IU is not significant. This is due to the greater losses by transpiration at 60 and 75% M.H.C. in the inoculated series being counterbalanced largely by the relatively smaller losses at 30 and 45%, as shown in

TABLE VII

MEAN LOGARITHMS OF DATA SUMMARIZED IN TABLE VI

M.H.C. of soil, %	Treatment	Periods								
		1	2	3	4	5	6	7	8	9
75	Uninoculated	2.803	3.199	3.383	3.461	3.509	3.322	3.395	3.048	1.971
75	Inoculated	2.671	3.035	3.304	3.480	3.580	3.431	3.515	3.287	2.737
60	Uninoculated	2.787	3.122	3.303	3.412	3.495	3.394	3.488	3.287	2.631
60	Inoculated	2.616	2.993	3.285	3.447	3.550	3.459	3.561	3.419	2.903
45	Uninoculated	2.582	2.912	2.974	3.020	3.151	3.128	3.258	3.151	2.735
45	Inoculated	2.351	2.761	2.948	2.897	2.973	2.937	3.097	3.041	2.694
30	Uninoculated	1.848	2.450	2.706	2.764	2.798	2.707	2.818	2.693	2.197
30	Inoculated	1.866	2.252	2.564	2.700	2.661	2.582	2.675	2.595	2.212

TABLE VIII

ANALYSIS OF VARIANCE OF LOGARITHMS OF DATA SUMMARIZED IN TABLE VI

Source of variance	Sum of squares	D.f.	Mean square
Replicates	0.036157	3	0.012052
Treatments	24.299005	7	3.471286**
Moistures	23.581827	3	7.860609**
IU	0.037721	1	0.037721
Moistures × IU	0.679457	3	0.226486**
Error I	0.890425	21	0.042401
Periods	22.517679	8	2.814710**
Periods × moistures	2.858726	24	0.119113**
Periods × IU	0.903290	8	0.112911**
Periods × moistures × IU	0.790471	24	0.032936**
Error II	1.196329	192	0.006231

Table IX. The highly significant interaction, Moistures × IU, leads to the conclusion, however, that inoculation does affect transpiration, and that the effects vary with different levels of soil moisture. At 60 and 75% M.H.C. inoculation accelerated transpiration, while at 30 and 45% M.H.C. inoculation retarded transpiration.

The interaction, Periods × IU, is highly significant. Fig. 2 indicates that transpiration in the inoculated plants was low in the early periods but at later periods tended to equal and even surpass that of the uninoculated plants. The second order interaction, Periods × Moisture × IU, which is also significant statistically, indicates that plants at all moistures did not respond in the same way to inoculation at all periods. This is clearly seen in Fig. 2, where the curves for transpiration in the inoculated plants at 30 and 45% M.H.C. do not rise above the uninoculated straight line curve as do those at 60 and 75% M.H.C. in the later periods. In this figure it appears that inoculated plants at 30 and 45% M.H.C. recovered at a slower rate than those at the higher moisture levels, but at about the period of maximum growth

the tendency toward recovery was lost, and the relative transpirational curves quickly reverted to approximately their original levels. The high portion of the curve for inoculated plants at 30% M.H.C. between 10 and 20 days after emergence is due to a wilted condition in the controls at this moisture level, which was much more pronounced than in inoculated plants.

Data on oven-dry weights of plants including roots, and the total water transpired were used to calculate the water requirements. These three sets of data are shown in Table IX. Table X gives the analyses of variance.

TABLE IX

DATA FROM EXPERIMENT III ON DRY WEIGHTS OF PLANT TOPS AND TOTAL WATER USED, AND WATER REQUIREMENTS

M.H.C. of soil, %	Treatment	Yield of dry matter, gm.	Water used per pot, gm.	Water requirements, gm.
75	Uninoculated	25.87	16619	643
75	Inoculated	29.07	19008	654
60	Uninoculated	33.72	17765	526
60	Inoculated	37.22	19719	529
45	Uninoculated	23.92	10039	421
45	Inoculated	19.35	7433	401
30	Uninoculated	11.02	3931	355
30	Inoculated	8.50	3055	358

TABLE X

SUMMARY OF ANALYSES OF VARIANCE ON THE THREE SETS OF DATA OF TABLE IX

Source of variance	D.f.	Mean square		
		Yield of dry matter	Water used	Water requirements
Replicates	3	6.089	1118925	1595.5
Treatments	7	421.123**	190191371**	57958.3**
Moistures	3	942.656**	432392608**	134871.8**
IU	1	0.980	369800	5.3
Moistures × IU	3	39.637*	11263658*	362.4
Error	21	10.938	2820465	664.8

The yields of dry matter and the amounts of water transpired were not influenced appreciably, as shown by the mean variances for inoculation with *H. sativum*. However, an examination of Table IX indicates different effects produced by inoculation at the different levels of soil moisture. Furthermore, the interaction of moistures and inoculation gives values that lie between the 5% and 1% levels of significance. Thus, it appears that yields of dry matter and water transpired were significantly increased by inoculation at the higher levels of soil moisture, and significantly reduced at the lower levels. The water requirement was very greatly influenced by soil moisture levels, but

inoculation had no significant effect in this experiment. No significance can be attached to the interaction effect here, indicating that the interaction effects observed for yield of dry matter and total water consumption are closely related.

Transpirational area measurements were made at three stages of development of the plants in this experiment. Three plants were selected at random for these records. Measurements were made on March 8 and 24, and May 9. By the latter date a number of the lower leaves had dried up. They varied from five to six at the lower soil moisture levels to about seven or eight at the 75% soil moisture level.

The total water losses for periods of six days, centering on each of the dates of measurement, were taken from the records of transpiration, given in summarized form in Table VI. From these and data on transpiring areas (Table XI) the rates of water loss were calculated (Table XII). The data on rates of transpiration were subjected to analysis of variance (Table XIII).

Table XI shows the greatest reduction in areas at the first date of measurement. Expressing the areas of the inoculated plants as percentages of the areas of uninoculated plants regardless of soil moistures, they are 56, 71,

TABLE XI

TRANSPIRING AREAS PER PLANT

M.H.C. of soil, %	Treatment	Transpiring areas, sq. cm.		
		March 8	March 24	May 9
75	Uninoculated	65	262	151
75	Inoculated	39	224	210
60	Uninoculated	62	303	262
60	Inoculated	36	204	279
45	Uninoculated	42	191	128
45	Inoculated	19	114	123
30	Uninoculated	19	72	96
30	Inoculated	12	51	94

TABLE XII

RATE OF TRANSPIRATION IN EXPERIMENT III

M.H.C. of soil, %	Treatment	Mg. per sq. dm. per hr.		
		March 8	March 24	May 9
75	Uninoculated	462	320	626
75	Inoculated	591	284	630
60	Uninoculated	471	238	464
60	Inoculated	547	325	503
45	Uninoculated	443	221	572
45	Inoculated	549	284	457
30	Uninoculated	182	237	297
30	Inoculated	272	235	218

TABLE XIII

ANALYSIS OF VARIANCE OF RATES OF TRANSPIRATION IN TABLE XII

Sources of variance	D.f.	Mean squares
Replicates	3	14528
Treatments	7	120634**
Moisture	3	269748**
IU	1	21570
Moistures × IU	3	4542
Error I	21	6420
Periods	2	381579**
Periods × Moistures	6	46076**
Periods × IU	2	37811**
Periods × Moistures × IU	6	6608
Error II	48	4925

and 111% for the three dates, respectively. On May 9 the areas of inoculated plants at 60 and 75% M.H.C. of the soil were greater than in the uninoculated plants, while at 30 and 45% M.H.C. they were slightly less. These facts are correlated with those shown by the transpirational histories in Table VI and Fig. 2.

Combining the data on areas with water loss to derive rates of transpiration as in Table XII, however, it becomes evident that the intensity of transpiration varies with the different treatments. In addition to the obvious effects of periods there are highly significant effects due to soil moistures and the inter-

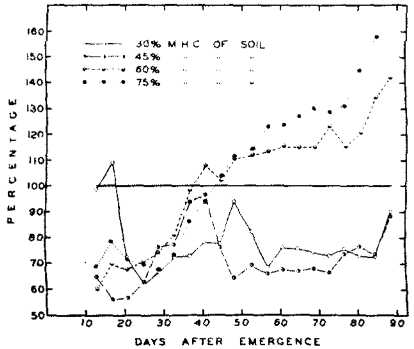

FIG. 2. *Transpirational histories of wheat inoculated with Helminthosporium sativum. Water losses over four-day periods are shown as percentages of the water losses of uninoculated plants. The horizontal straight line at the 100% point represents the water used by the controls. Comparisons are shown for soil moisture maintained at 30, 45, 60, and 75% M.H.C. of the soil.*

action of periods with moistures. Inoculation significantly increased the rate of transpiration during the first period. This effect was less with each succeeding period, resulting in the significant interaction, Periods × IU.

The average areas of the successively appearing leaves indicate the mechanism by which inoculated plants produced greater yields than the controls. In Fig. 3 the average areas of leaves of inoculated plants (black bars) are compared with those of control plants. Inoculation very markedly reduced

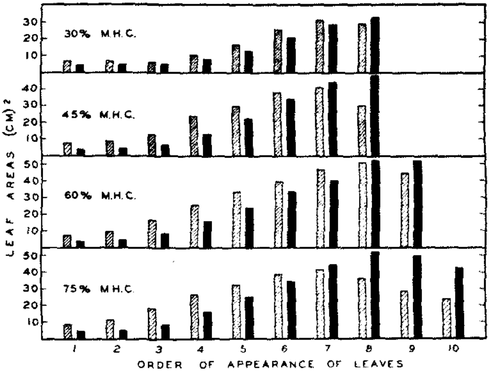

FIG. 3. *Bar diagrams showing transpiring areas in square centimetres of leaves on the main culms, in their order of appearance from inoculated wheat plants (black bars) compared with those from uninoculated plants (diagonally marked bars). The comparisons are made at soil moisture levels of 30, 45, 60, and 75% M.H.C. of the soil.*

the areas of the leaves up to about the sixth or seventh to appear. The later leaves to appear were larger in the inoculated plants, particularly at 75% M.H.C. As the earlier leaves died successively the total transpiring areas in the inoculated plants became more nearly equal to those of uninoculated plants, and eventually became greater as the process went on. Thus, during the flowering and filling stages the products of photosynthesis in the inoculated plants at the higher soil moistures were probably greater than in the control, accounting for increased yields. At the lower soil moistures the same factors were at work. However, the recovery was not sufficiently rapid to show the same beneficial results in increased yields. In Fig. 3 the difference between inoculated and controls at 60% M.H.C. will not account for the increased yields in the inoculated plants. In this case more tillers and more main culm leaves in the inoculated plants were sufficient to account for the increased yields.

Fig. 4 was prepared from the lengths and widths of the leaves of the main culms to show how the leaf areas were first reduced and then increased in

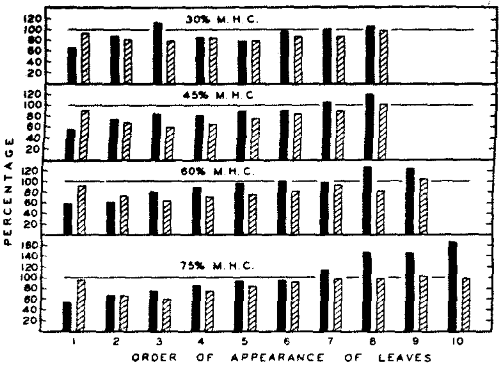

FIG. 4. *Bar diagrams showing leaf lengths (black bars) and leaf widths (diagonally marked bars) of wheat inoculated with Helminthosporium sativum as percentages of leaf lengths and widths of control-plant leaves. The plants were grown at four levels of soil moisture.*

the inoculated plants compared with the controls. The black bars represent leaf lengths and the diagonally marked bars represent leaf widths of the inoculated plants expressed in percentage of the lengths and widths of control plant leaves.

The first leaf to appear was reduced in width very slightly by inoculation, but it was reduced in length to 60% or less of the control. Successive leaves in the inoculated plants improved in length until the later ones exceeded those of the controls. In width the leaves of inoculated plants dropped from about normal in the first leaf to about 60% of normal in the third leaf. In succeeding leaves width improved to normal. However, it is interesting to note that the increased areas of the later leaves of inoculated plants (Fig. 3) were due entirely to increased lengths, while the widths remained as in the controls.

Experiment IV

This experiment was conducted at Saskatoon from December 12, 1937, to March 24, 1938. The soil was autoclaved at 15 lb. pressure for 2 hr. The treatments were as in Experiment III. Evaporation from the soil was largely prevented by covering the surface with a mixture of fine sand and petrolatum. This was packed firmly around the bases of the plants and edges of the containers. The seedlings were thinned to three in each pot. Each replicate was placed in a block in a permanent position on the greenhouse bench. The four blocks were arranged in a 2 × 2 square. At each weighing the positions of pots within the blocks were randomized. Since the experiment was begun in a period of short days, artificial light was supplied from 5 p.m. to 8 a.m.

throughout the course of the experiment. This added light was insufficient, however, to produce good growth.

The transpirational data were assembled into ten periods of ten days each, and are presented in Table XIV. An analysis of variance is given in Table XV.

TABLE XIV

DATA ON TRANSPIRATION FOR EXPERIMENT IV

M.H.C. of soil, %	Treatment	Periods									
		1	2	3	4	5	6	7	8	9	10
75	Uninoculated	19	48	110	180	217	240	299	252	233	185
75	Inoculated	12	31	80	131	173	209	206	187	150	144
60	Uninoculated	18	39	79	124	142	182	210	204	186	141
60	Inoculated	12	29	67	112	132	177	202	169	162	122
45	Uninoculated	11	22	38	59	74	116	111	86	64	41
45	Inoculated	8	14	28	50	66	96	89	82	61	31
30	Uninoculated	11	15	18	25	26	31	21	15	10	0
30	Inoculated	8	13	16	25	25	32	24	16	11	0

TABLE XV

ANALYSIS OF VARIANCE OF LOGARITHMS OF DATA SUMMARIZED IN TABLE XIV

Source of variance	Sums of squares	D.f.	Mean squares
Replicates	1.412803	3	0.470934**
Treatments	47.844885	7	6.834983**
Moistures	47.137578	3	15.712526**
IU	0.548633	1	0.548633**
Moistures × IU	0.158673	3	0.052891
Error I	0.562024	21	0.026763
Periods	28.006400	9	3.111822**
Periods × Moistures	14.528706	27	0.538100**
Periods × IU	0.130970	9	0.014552
Periods × Moistures × IU	0.041686	27	0.001544
Error II	3.180747	216	0.014726

The results of this experiment are comparable with those of Experiment II, in which light conditions were unfavourable for good growth of the plants also. The mean variance for IU is highly significant while those for Periods × IU and the second order interaction are non-significant. These facts indicate that inoculation was effective in reducing transpiration, and that there was no marked tendency under the conditions of the experiment for inoculated plants to recover.

Oven-dry weight of plants including the roots and the total water transpired are given in Table XVI. From these two sets of data the water used per gram of dry matter was calculated for each unit of the test. These data also are presented in Table XVI. Table XVII shows the analyses of variance.

TABLE XVI

DATA FROM EXPERIMENT IV ON DRY WEIGHTS OF PLANTS, TOTAL WATER USED PER CONTAINER AND WATER REQUIREMENTS

M.H.C. of soil, %	Treatment	Yield of dry matter, gm.	Water used per container, gm.	Water requirements, gm.
75	Uninoculated	2.84	1783	638
75	Inoculated	1.96	1323	678
60	Uninoculated	2.23	1325	604
60	Inoculated	1.82	1184	654
45	Uninoculated	1.02	622	612
45	Inoculated	0.86	525	614
30	Uninoculated	0.86	172	453
30	Inoculated	0.38	170	456

TABLE XVII

ANALYSES OF VARIANCE OF DATA SUMMARIZED IN TABLE XVI

Source of variance	D.f.	Mean variances		
		Yield of dry matter	Water used per container	Water requirements
Replicates	3	0.7628**	165887**	5276*
Treatments	7	3.3070**	1427772**	29843**
Moistures	3	7.0669**	3170783**	66898**
IU	1	1.0658**	245350**	4465
Moistures × IU	3	0.2942	78901	1247
Error	21	0.1084	29981	1584

While the total water used per container was markedly affected in this experiment by inoculation with *H. sativum*, the water requirement was not significantly influenced. The interaction, Moisture × IU, was not significant either for yield of dry matter or for total water transpired per container. Thus, it appears that in this experiment, conducted under poor light conditions, there was no tendency at the higher soil moisture levels for inoculated plants to produce as much dry matter as uninoculated plants.

Transpiring areas were calculated from measurements made on all leaves and stems of all plants in this experiment on December 24, January 5, 17, and 31. Table XVIII shows the average transpiring areas per plant for the various treatments at the four dates of determination in square centimetres.

The total water transpired from each flask for eight-day periods centering on each of these dates was used to calculate the rates of transpiration. These are given in Table XIX and the analysis of variance in Table XX.

As in Experiment III the transpirational areas of inoculated plants are reduced to 60% or less of those in the uninoculated plants, at the first time of measurement. However, unlike Experiment III there was only slight

TABLE XVIII

Transpiring areas (sq. cm.) per plant, in Experiment IV

M.H.C. of soil, %	Treatment	Dec. 24	Jan. 5	Jan. 17	Jan. 31
75	Uninoculated	18.8	46.8	72.3	98.0
75	Inoculated	10.8	27.0	51.4	63.0
60	Uninoculated	19.0	41.6	61.3	66.4
60	Inoculated	11.2	29.7	52.1	62.2
45	Uninoculated	16.6	36.1	47.3	44.4
45	Inoculated	9.6	20.3	34.1	32.6
30	Uninoculated	16.5	30.9	31.1	21.6
30	Inoculated	9.9	20.1	30.2	19.6

TABLE XIX

Rate of transpiration in Experiment IV

M.H.C. of soil, %	Treatment	Mg. per sq. dm. per hr.			
		Dec. 24	Jan. 5	Jan. 17	Jan. 31
75	Uninoculated	203	241	307	262
75	Inoculated	219	269	299	317
60	Uninoculated	168	196	253	242
60	Inoculated	198	221	271	252
45	Uninoculated	120	110	163	198
45	Inoculated	136	142	188	242
30	Uninoculated	110	78	101	146
30	Inoculated	139	99	104	159

TABLE XX

Analysis of variance of rates of transpiration, Table XIX

Source of variance	D.f.	Mean squares
Replicates	3	624.0
Treatments	7	61221.7**
Moistures	3	137288.4**
IU	1	15975.8**
Moistures × IU	3	236.9
Error I	21	842.0
Periods	3	32149.3**
Periods × Moistures	9	4626.3**
Periods × IU	3	638.8
Periods × Moistures × IU	9	461.1
Error II	72	398.0

recovery in succeeding periods. This agrees with the transpirational histories (Table XIV), in which the failure of the inoculated plants to recover fully, even at the higher soil moistures, was attributable to poor light conditions.

Rates of transpiration, Table XIX, are significantly greater in the inoculated than in the uninoculated plants as in Experiment III. On the other hand the non-significant interaction, Periods × IU, indicates the failure of the plants to recover. There was an average of 12% more water transpired per unit area by inoculated plants than by uninoculated plants. This varied only slightly from period to period and from one soil moisture level to another, yet the 4% increase in the water requirements due to inoculation failed to reach the level of significance. There may have been an increased production of dry matter per unit of leaf area which kept down the water requirements in spite of the increased rate of transpiration.

Experiment V

This experiment, conducted at Madison, Wis., was designed to determine whether inoculated seedlings had higher or lower water requirements than uninoculated seedlings. The data are summarized in Table XXI and the analyses of variance are given in Table XXII.

TABLE XXI

Dry weights of tops and roots of wheat in Experiment V, weight of water transpired and water requirements

M.H.C. of soil, %	Treatment	Dry weight, mg.		Water transpired per plant, gm.	Water requirements, gm.
		Tops	Roots		
75	Uninoculated	68.2	10.8	46.6	590
75	Inoculated	64.2	10.6	46.1	616
60	Uninoculated	77.2	12.6	48.7	541
60	Inoculated	62.2	10.8	40.4	555
45	Uninoculated	63.2	15.8	32.9	417
45	Inoculated	51.4	13.4	25.8	399
30	Uninoculated	42.4	17.4	12.6	211
30	Inoculated	38.2	14.2	13.8	262

TABLE XXII

Analyses of variance of data summarized in Table XXI

Source of variance	D.f.	Mean squares			
		Dry weight		Water transpired per plant	Water requirements
		Tops	Roots		
Replicates	4	134.1	12.79	116.74*	2094
Treatments	7	881.2**	31.49**	1063.66**	113965**
Moistures	3	1724.5**	57.40**	2386.01**	262234**
IU	1	765.6**	36.10*	129.24	4452
Moistures × IU	3	76.4	4.03	52.79	2201
Error	28	58.9	6.96	41.19	1218

While significant reductions in dry weight of tops and roots of the seedlings were produced by *H. sativum*, particularly at 60 and 45% M.H.C. of the soil, the water transpired and the water requirements were not significantly influenced. The increase in water requirements due to inoculation at 30% M.H.C. is explainable by the fact that wilting was apparent in the control seedlings at this moisture level two to three weeks after emergence. Root development at the lower soil moisture levels was significantly greater than at the higher.

Discussion

The central fact brought out in this work is the recovery of plants inoculated with *H. sativum* after an initial severe set-back in the seedling stage. Inoculation of the seed with the fungus before sowing ensured an immediate attack on the seedlings during and immediately following germination. A retardation in emergence of one to two days occurred in all experiments. Rates of transpiration, leaf areas, dry weights, and disease ratings all indicated a poor development of the inoculated seedlings. The inoculated seedlings were reduced to as low as 50% or less of the controls in leaf areas and transpiration.

Under favourable conditions the retarded seedlings showed definite signs of recovery as indicated by the transpirational curves in relation to the controls and by relatively increasing sizes in successively appearing leaves. That the recovery is more than mere catching up to the controls is indicated by an actually greater use of water, greater leaf areas, and increased yield in the inoculated as compared with control plants.

That conditions of growth must be favourable to the plant before recovery can occur is indicated by Experiments II and IV, in which light conditions were poor, and by Experiment III, in which recovery was permanent only at the higher soil moisture levels. Slow recovery appeared in the latter experiment at 30 and 45% M.H.C. of the soil. At about the time of elongation of the culms, however, the tendency to recover was suddenly lost as indicated by the transpirational history, and the plants reverted to about the same level as in the seedling stage, in their relative use of water.

The recovery of the inoculated plants from the initial set-back was attended by a more rapid appearance of leaves, the formation of more leaves, and greater areas in the later appearing leaves. As the plants approached the flowering and later stages, the lower leaves of both inoculated and uninoculated plants died in succession. With the loss of the lower stunted leaves, the total functional leaf areas of the inoculated plants became greater than in the controls. The greater photosynthesis in consequence, would account for the increased yields that were observed.

The initial set-back following infection of wheat by *H. sativum* may be due to a toxin, in view of the findings of Vanterpool (10). He reports that *H. sativum* grown on wheat-grain medium produced a water-soluble toxin that caused slight retardation of the plumules and moderate shortening of the roots in germinating wheat. What the influence of the toxin may be on the plant to cause stunting is obscure. Possibly the formation of growth promot-

ing substances or their distribution in the plant may be adversely affected. In this connection it is interesting to note that the first appearing leaves are not reduced in width appreciably but are decidedly reduced in length. The second and third appearing leaves are decidedly reduced in width as well as in length. Here the reduced activity of growth substances may be accompanied by a reduction in nutrition due to smaller photosynthetic areas.

On the other hand, a saprophytic development of the fungus upon the endosperm or upon the aleurone layer, increasing in effect during the development of the first and second foliage leaves, may account initially for the reduced size of these organs. The close vascular connections between the first two foliage leaves and the scutellum has been pointed out by McCall (6). The reduced size and consequently lessened photosynthetic activity in addition to the diversion of food stores in the endosperm by the fungus results in small growth in the third, fourth, and fifth leaves. Without the intervention of some other factor this condition would continue as long as leaves were formed, transpiration would continue to be small, and final yields of dry matter would be reduced from that of the controls. Some factor must be present tending toward recovery, which under favourable conditions for growth results in increased rather than decreased yield and consumption of water.

How the inoculated plants with the first five or six leaves decidedly stunted, compared with control plants, were able to produce further leaves that were larger than corresponding leaves in the controls, in view of their reduced photosynthetic areas, constitutes an interesting problem. Maximov (5) reviewed the works of Zalenski in Russia and Yapp in England, in which the upper leaves of plants were shown to be more xeromorphic than the lower ones. This is indicated by greater total length of vascular bundles and more stomata per unit area of leaf surface, and smaller dimensions of epidermal cells. These findings were confirmed by Heuser for wheat. Maximov's colleague Alexandrov demonstrated that the intensity of transpiration increases from the lower leaves to higher leaves on the same plant. Zalenski has shown that the more xeromorphic condition of the upper leaves is due to the deflection of water from the growing tip by the fully developed and transpiring leaves. By removing the expanded leaves of *Coleus* he found that subsequently developed leaves resembled lower leaves in a looser network of veins, reduced number of stomata per unit area, and increased size of cells.

In the recovery of inoculated wheat seedlings it may be that toxin development by the fungus either ceases or the concentration of toxins is reduced below injurious concentrations owing to increasing size in the plant so that normal growth is resumed. The reduced lower leaves divert less water from the growing point and the expanding leaves consequently are less xeromorphic and larger than in the control plants. The failure of inoculated plants at 30 and 45% M.H.C. of the soil to show recovery in the same degree probably is due to the inability of the plants to supply water rapidly enough to prevent the new leaves becoming highly xeromorphic in structure.

Another possible explanation may be mentioned. Sande-Bakhuyzen (9) states, "There is evidence in the literature that the favorable effect of supplying plants with mineral salts within certain limits does not increase the photosynthesis rate, but increases leaf area, causing increased yield. This probably means a lengthening period of leaf formation, i.e., more (and larger) leaves and consequently more photosynthates." The toxic or other influences produced by the growth of *H. sativum* in the host tissues may increase the rate of intake of mineral ions into the plant, and as the effects of the deleterious substances decrease to allow recovery, the presence of large amounts of minerals may influence the sizes of subsequently formed leaves.

No significant increase was observed in the water requirements of plants in the seedling stage owing to inoculation with *H. sativum*. However, the rate of transpiration in the seedling stage per unit of transpirational area was found to be significantly greater in the inoculated plants. To reconcile these two conclusions a more intense photosynthetic activity by the smaller leaves of the inoculated seedlings may be postulated. Miller (7) considered that one of the chief causes of higher transpiration rates in plants with small leaf surface is their ability to maintain a more saturated condition of the external cell walls of the leaves than plants with larger surface.

The application of the results obtained in the greenhouse to field conditions must be made with caution. One very important difference between the experiments reported in this paper and the course of events in the fields of Western Canada is in the time of infection. The seed sown in the field generally has only a relatively small proportion of the seeds bearing the fungus, and infection from the soil increases most rapidly as soil temperatures rise above 15° C. one to two months after seeding. There is, however, evidence in the author's unpublished work that the plants showing severe lesions of the subcrown internodes were stunted as to height, and these plants show definitely reduced yields as compared to neighbouring plants (8). There appears to be an effect on the plant similar to that found in the greenhouse, but owing to the delayed infection there may not be the same tendency to recover, or the time between infection and maturation of the plants may be too short for recovery to be marked. There is also the problem of competition. In the greenhouse experiments no competition for soil moisture and nutrients occurred between the uninoculated and inoculated plants. In the field, on the other hand, diseased plants are subjected to intense competitive conditions, from slightly affected or unaffected wheat and weeds. Under Western Canadian conditions the precipitation is small and soil moistures are usually relatively low. As shown in the greenhouse, low soil moisture levels did not permit inoculated plants to recover normally. It appears, therefore, from these considerations that complete recovery of plants from an initial setback owing to infection with *H. sativum* in the field is unlikely.

Acknowledgments

The author wishes to acknowledge indebtedness to Dr. P. M. Simmonds and Mr. R. J. Ledingham, who first delved into the influence of root-rotting fungi on water relations in wheat and turned his attention to the problems involved. To Dr. J. G. Dickson, of the University of Wisconsin, goes the author's gratitude for many helpful discussions of the work during its progress. The Dominion Department of Agriculture kindly made it possible for the writer to conduct certain phases of this work at the University of Wisconsin.

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STUDIES ON THE INK-SPOT DISEASE OF POPLAR[1]

By Réne Pomerleau[2]

Abstract

A foliage disease of poplar caused by *Sclerotinia bifrons* (E. & E.) Whetzel has been studied during an important outbreak in the years 1935–1937. The perfect stage of this fungus has been correctly named by Whetzel. The fungus hibernates on the ground in the sclerotial condition, and during the spring apothecia are produced on sclerotia. Inoculation of the leaves is induced by ascospores ejected from apothecia. After two or three weeks of incubation, reddish areas appear on leaves; new sclerotia are formed in the lesions in June and are ready to fall about July 15. The epiphytotic development of the disease is favoured by the following set of conditions: presence of a fairly large number of sclerotia on the ground, occurrence of a dense stand of young poplars, low temperature and high humidity before and at the time of foliation. Quite a number of trees at the sapling stage are killed and others are affected by an intense defoliation, during an outbreak. These effects are noticeable only in thickets of pure population of Aspen, which represent usually the first stage of natural reforestation under Quebec conditions.

Introduction

In 1936, special climatic conditions in the province of Quebec, doubtless also in the neighbouring provinces and the adjacent part of the United States, favoured the development of a fungus affecting poplars, and consequently a fairly important outbreak resulted. The appropriate name "Ink-spot disease of poplar" was used a few years ago to designate the disease. Although this is widely spread on the North American continent, it does not appear, under normal conditions, to be very injurious to the trees. This epiphytotic started in 1935 and reached its climax in 1936, to regress markedly in 1937.

The wide occurrence of the disease enabled the writer to study its development and its effect on poplar; a certain number of interesting and unpublished facts regarding the biology and ecology of the parasite were also noted. In this article are discussed the results of a study of this quite unknown fungus. Material was collected for the histological study of the diseased leaf and the cytological investigation of the pathogen, but these will be reported in another paper.

In the following pages, an historical sketch of the disease is accompanied by notes regarding its range, importance, and the species affected; it is followed by a discussion of the identity of the fungus and a study of the biology of the pathogen, of the factors favouring its development, and, finally, by some observations on its pathological effects.

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Contribution from the Laboratory of Forest Pathology, Department of Lands and Forests, Quebec.
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History

The ink-spot disease of poplar has been known in the United States and Canada for a good many years. As early as 1890, Ellis and Everhart applied the name *Sclerotium bifrons* to the imperfect stage of the fungus in question.

Although its occurrence had been recorded in New England, in the Canadian Rockies, and elsewhere, its perfect stage was unknown until recently. In 1920, Hartley and Hahn (6) pointed out that sclerotia had been collected in various places in the United States and Canada, but that sexual fruit bodies were not observed. Overholts (7) reported the fungus from Colorado, but he has found no sign of fruiting bodies. The first mention of the perfect stage of *S. bifrons* was made by Seaver and Shope (11). These authors state that the apothecia arising from sclerotia on the ground were found in abundance at the foot of poplars in Colorado. They state also that the name "Ink-spot fungus" was given by Dr. T. D. A. Cockerell. Later, some authors used the name *Sclerotinia bifrons* (Ellis & Everhart) Seaver & Shope to designate the organism. It will be seen later that their use of this binomial was an error.

In 1935, the writer reported (8) the presence of the fungus on different species of poplars in Quebec and finally, in 1936, it was held (9) responsible for a fairly important outbreak on aspen.

Range and Importance

The above historical sketch shows that this fungus is an American endemic species occurring almost everywhere in the United States and Canada in association with *P. tremuloides*. As this species is distributed from one ocean to the other in Canada, in the northern states and the mountain regions of western United States, it may be said that *S. bifrons* is spread throughout the whole temperate region of the North American Continent.

The phenological observations presented in this paper, coupled with those of Overholts (7), show, however, that the organism prefers the fairly cold climates. Moreover, in such states as Colorado and Wyoming, it was observed only in the mountain regions. Hartley and Hahn (6) report that in 1920, in the collection of the Department of Forest Pathology in Washington, there were 20 collections of the fungus, 15 of which were made by Dr. G. G. Hedgcock in Colorado, and five in New England and New York State by Dr. Spaulding, G. L. Barrus, and themselves. Other collections from Wisconsin, Utah, Ontario, and Virginia are also mentioned.

In Quebec, it was found almost everywhere. Previous to 1935, severely affected stands of *P. tremuloides* were rather rare. Occasionally a few Lombardy poplars growing in damp and cold places showed a fairly large number of diseased leaves. But in 1935 the writer observed a rather bad attack, especially in the vicinity of Berthierville. In the same year, a fairly severe epiphytotic was reported from the vicinity of Rimouski, where about 50% of the leaves in a stand of *P. tremuloides* were killed. However, it was in

1936 that the parasite developed in an unusual way. During that year, the presence of the disease in the epiphytotic condition was reported from the four corners of Quebec, and it was ascertained by personal visits that in certain young stands not infrequently more than 75% of the foliage was affected. In a fairly large number of cases, an almost complete defoliation had resulted from the spread of the pathogen, thus bringing about the death of young individuals.

Species Affected

The occurrence of this parasitic fungus on *P. tremuloides* and *P. nigra* has been known for many years. It appears as if the former species were the chief suscept of the pathogen. Dr. Spaulding, as reported by Hartley and Hahn, has noted "that in mixed stands of *P. tremuloides* and *P. grandidentata* only the former is attacked," in the Adirondacks. The author noted this fact also several times. In 1936, in Berthierville, there were a large number of *P. grandidentata* entirely free from the disease, whereas the trembling aspens were very badly affected. On June 22, 1937, several individuals of *P. grandidentata* were found in a fairly damp forest of Parks Reserve (Kamouraska County) with the leaves infected and bearing sclerotia of *Sclerotium bifrons*.

In 1937, the author reported (9) the discovery of the fungus on a leaf of the Carolina poplar (*P. canadensis*) in Berthierville (Plate I: 1). There appears to be no other species of poplars on which the fungus was recorded. Perhaps some unpublished data might increase the list of suscepts of the parasite.

Identity of the Pathogen

The name *Sclerotium bifrons* was used until 1930, when Seaver and Shope (11) proposed the combination *Sclerotinia bifrons* for the fructification they found on sclerotia in Colorado. This binomial has since been used at least twice in text-books to designate the fungus, for the stipitate apothecia arising from the sclerotium belong to the *Sclerotinia* type (11).

However, the question arises as to whether the organism reported by Ellis and Everhart is exactly the same as the one found by Seaver and Shope. Professor Whetzel, who made a thorough study of the species of the genus *Sclerotinia*, expressed some doubts as to the identity of the fructifications found in Colorado. Morphological studies of the perfect stage of the fungus collected at Berthierville extended over two seasons, but as a paper on this species will be published soon by Dr. Whetzel, there is no need here of a long discussion on the matter.*

It is felt that the species described and illustrated by Seaver and Shope cannot be homologated with the organism that the author found growing from sclerotia formed on poplar leaves. From a comparison of Plate I; 7, with

* *Since the present paper was written, Whetzel (12) has described the perfect stage of the true causal organism, giving the history of its discovery and the correct name. According to an article of the International Rules of Botanical Nomenclature, F. J. Seaver, in the same number, rejects the name S. bifrons Whetzel and proposes S. Whetzelii for the species on eastern poplars.*

figures given by Seaver and Shope, discrepancies can be easily detected. By colour and measurements these differences are also quite noticeable. Characters of the asci (Fig. 1) cannot fit for both organisms.

Ellis and Everhart designated this fungus under the name *Sclerotium bifrons*, after a collection made by Dearness in London, Ontario, but they gave no description. The type-collection is No. 2554 of the "North American Fungi". Saccardo (10) gives the following diagnosis: "167. *Sclerotium bifrons* Ell. et Ev. N.A.F. n. 2554 (absque diagnosi).—Stromatibus sparsis, amphigeno-bifrontibus, discoideis, latis, purpureo-nigris, tenuiter rugulosis, 4–6 mm. diam., intus albidoroseis, tandem excisis et foramina in foliis relinquentibus. Hab. in foliis dejectis Populii, London, Canada, 1890. (Dearness)."

This diagnosis fits well the material collected here. It appears logical, however, that this description be completed since the perfect stage is known. Publication of these notes was delayed until Professor Whetzel's description of the perfect stage, in which he gives a correct name to the organism, had appeared. To complete the present work, however, the writer's own description is given, based on the material collected in Berthierville (Quebec):

Sclerotia (4-6 × 2-4 mm. diam., 0.25–0.50 mm. thick) scattered on the leaves, orbicular, oblong, sometimes reniform and irregular, easily separable from the leaf at maturity and leaving a hole in falling, black brown, slightly rugose, white inside, slightly convex on the upper surface and concave on the lower one. Apothecia (4–6 mm. diam.) 1 to 3 per sclerotium, at first cupuliform, becoming almost plane at maturity, dark brown, with long stipe. Stipe up to 15 mm. in length, slender, brown, darker toward the base. Asci (130–160 × 10–13 μ) cylindrical, gradually attenuated at the base, truncate at the apex; ascospores (10–13 × 5-7μ) obliquely uniseriate, unicellular, oblong-ellipsoid, biguttulate, hyaline, with granular content; paraphysis abundant, filiform, septate, with clavate apex. (Fig. 1.)

It is not merely from the morphological standpoint that the identity of the fungus is hard to establish. Biologically also it presents some difficulties. Thus, in trying to cultivate it artificially, whether by sowing the spores or by planting a piece of the thallus on nutrient medium, the results are always negative. However, cultures develop occasionally from the ascospores of certain apothecia that form under poplar trees; these grow actively and finally produce sclerotia in abundance (Plate 1; 3). The asci have about the same shape as those of the sclerotia found on the infected leaves. In the spring of 1933, an active culture was obtained by planting debris of apparently frostbitten young poplar leaves on potato agar. In 1936, more cultures were obtained by spore discharge of fructifications on agar. On the other hand, numerous attempts were unsuccessful with certain fruit bodies well attached to the sclerotia, as were attempts to inoculate living leaves of poplar with mycelium from artificial cultures.

Careful examination of the fructifications found on the ground in the spring shows that some of them differ from the others, in that they are smaller and attached to a sclerotium embedded in the debris of leaves. In 1933,

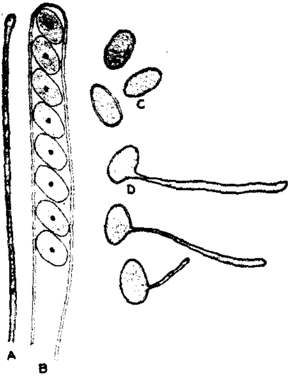

FIG. 1. *Sclerotinia bifrons (E. & E.) Whetzel. A. Paraphysis; B. Fertile portion of the mature ascus; C. Ascospores; D. Germinating ascospores* (× 500).

specimens were sent to Professor Whetzel, who stated in personal communications that, in unpublished work, he applies the name *Sclerotinia pseudobifrons* to this saprophytic species, which occurs almost always among the dead leaves of poplars affected by *Sclerotium bifrons*. The writer's observations on this form agree with those of Dr. Whetzel. The culture obtained in 1933, from dead parts of partially frozen living leaves of Lombardy poplar, should be regarded also as belonging to this species.

Another aspect of the problem of the identity of the fungus and of its developmental forms deserves mention. Davis (2) pointed out the presence of an imperfect fungus, *Myrioconium comitatum*, associated with *Sclerotium bifrons* and *Sclerotium foliicola* on willow. Davidson and Cash (1) believe that there is reason to consider it as being the imperfect stage of some species of *Sclerotinia*. As this stage was not found in the course of the present study, neither its authenticity nor its relations to the perfect stage are contested.

Biological Observations

A fairly important infection observed in a young mixed stand of aspens (*P. tremuloides*) and old field birches in the forestry nursery of Berthierville, in 1935, gave the writer the opportunity to study this disease, which was then almost unknown, and to describe the life cycle of the causal agent. During that year, a large number of leaves bearing spots and sclerotia were collected before their fall. These leaves were placed on the soil and covered with a screen to prevent their dispersion until the following spring.

The sclerotia were thus readily available while being kept under natural conditions, which enabled the writer to follow the different developmental stages of the fungus from the beginning of the season of vegetation in 1936. In that spring, however, the sclerotia of the previous year that had been disseminated naturally were numerous enough on the ground to be found without much difficulty.

Hibernation

The only organ of preservation of the parasite is, according to all evidence, the sclerotium, since it is the only portion of the organism that survives in the fall. Should the existence of an imperfect stage be established, this stage might play a role equivalent to the microspores of *Sclerotinia Gladioli* (4), but nothing is known of their capacity to serve as inoculum in the spring. It will be seen, however, that the ascospores alone suffice to bring about the infection when the environmental conditions are favourable.

Fructification

The author tried to obtain fructifications artificially on several occasions. Some sclerotia were placed in damp sand and kept at a fairly cold temperature in an electric refrigerator for a longer or a shorter period of time. When the sclerotia had not been allowed to overwinter under the snow, the results were negative. Consequently, the sclerotia collected in the summer or the autumn that follows their formation are not able to fruit. This is true also of those that passed the winter elsewhere than under the snow, for example, in the laboratory.

But sclerotia normally overwintered under the snow behaved differently: when placed in the refrigerator at a temperature slightly above the freezing point, they fruited abundantly very early in the spring, although the development of the fructifications on the margin of the sclerotia ceased as soon as they were brought to room temperature (Plate I; 10); when kept at room temperature on moist sand, they remained sterile.

From these laboratory experiments it appears that this fungus is able to form its sexual organs of reproduction only at relatively low temperatures and after overwintering of the sclerotia on the soil.

Outdoors, the fructifications are formed rather early in the spring, that is, a short time after the melting of the snow. Beginnings of ascophores were found as early as April 24, in 1936, but the development of the asci had not yet taken place. These organs, however, developed only on very damp soil and when protected from the sun by fairly dense shrubbery. Shortly thereafter, and coincident with the opening of the aspen buds, a large number of apothecia had formed and were ready to discharge their spores (Plate I; 6). This active phase of the parasite coincided with the foliation of the poplars, and occurred in Berthierville about May 7, in 1936. The fructifications developed much later in 1937. The first signs of their development appeared only about May 10, and the spores did not reach maturity before May 29,

though all the poplar leaves were expanded and had reached their full size some time previously.

Dissemination

As the fructifications of the fungus are formed on the soil, the spores must be discharged violently in order to bring about infections on the leaves, which are the only easily affected organs of the suscept. This ejection was brought about artificially by placing an apothecium rapidly in water without, however, covering the fertile surface. If the fruit bodies are matured, the spores then are discharged into the air with a light explosion that produces a cloudiness visible at a short distance from the ascigerous surface. According to this observation, it seems reasonable to believe that, in nature, the ascospores are ejected into the air when water moistens the fructifications during a period of rain. They are disseminated by the wind which brings them in contact with the leaves, according to the hydro-anemophile type of dissemination.

Germination

The ascophores collected and examined at the beginning of May, 1937, contained a large number of asci with well formed spores. These spores, when placed in a drop of water, germinated abundantly, and germ tubes 60 to 80 μ in length were observed after 20 hr. (Fig. 1). Although germination occurs easily in water, nevertheless the development of the mycelium does not exceed a certain stage on a substratum other than the poplar leaves. Thus, the spores discharged on potato agar germinated as well as in a drop of water, but the growth of the germ tubes soon came to a standstill.

Consequently, in nature the spores should germinate rapidly once transported to the poplar leaves, since the substratum is moistened with rain that brought about their ejection.

Inoculation

It was very easy to reproduce this part of the life cycle artificially in the laboratory. For successful inoculations it sufficed to atomize a suspension of spores on the surface of the leaves, and to maintain for 24 hr. the humidity of the latter by means of celluloid chambers built for this purpose (Plate I; 9). A few young potted trees, inoculated in this manner in the laboratory on May 13, showed on June 4 very conspicuous and characteristic spots on some of the leaves.

Infection

In 1936, the symptoms of the disease appeared on outdoor trees about June 5, a month after the opening of the buds. The pathological signs were sufficiently characteristic at that time not to be confused with those of other infections (Plate I; 2). They are, at the beginning, more or less extensive spots, which are separated clearly from the rest of the leaf tissue by reddish or dark brown discolorations. Not infrequently the whole leaf is thus discoloured. This change in the appearance of the affected leaves usually takes place very rapidly; in one or two nights the foliage of the trees may thus be

discoloured to an extent varying with the intensity of infection (Plate I; 2, 8). Usually the spots are circular or irregular; however, it frequently happens that the infected areas are narrow and linear (Plate I; 5). This seems to result from the fact that the mycelium develops only in those parts of the leaf that are abundantly moistened, as along a streak of water. Moisture is therefore a factor of primary importance upon which depends the severity of the infection. On the whole, the droplets or a film of water must remain on the leaves long enough to allow the penetration of the mycelium.

Most of the infected leaves showed symptoms of the disease between June 1 and 8. Practically no subsequent spreading of the lesions that had invaded the leaf blade at that time occurred. Thus, the growth of the mycelium in the leaf tissue is very rapid. This conclusion is substantiated by results from the inoculations carried on in the laboratory. It seems also that the extent of the infected area is dependent upon the degree of moisture available during the incubation period. Small foliated branches kept continually, from the time of inoculation until the appearance of symptoms, in a water-saturated atmosphere showed much larger spots than leaves left in the open air immediately after inoculation, that is, two or three days after the atomizing of the spores.

The temperature factor, aside from conditioning the development of the fructifications, seems to influence also the growth of the mycelium in the leaf. Thus, it was found that infections developed in the laboratory and at a higher temperature were not as severe as those developed in the open; both were inoculated at the same time and in the same manner. Also the development of the disease in the open is inhibited by the rise in temperature with advance of the season. This fungus is, therefore, a parasite inhabiting cold regions and developing early in the spring.

Formation of the Sclerotia

The infection period, including incubation and appearance of the lesions, was practically completed by June 15, 1936; as the temperature of the air became fairly warm, all growth ceased, except for the formation of the organs of preservation, the sclerotia, which marks the end of the vegetative activity for the year. The sclerotia showed on June 15 the first evidences of their formation. Felt and Rankin (5) state that the sclerotia of this fungus develop on the dead leaves in the fall. This is obviously incorrect because, on July 15, in Berthierville, the sclerotia are not only formed, but very often completely mature and lying on the ground.

At the beginning of this stage, one or several pale, circular or oblong spots may be seen in the infected portion of the leaf. Examined with transmitted light, these regions are paler than the rest of the leaf. They are the very places where the sclerotia arise somewhat later. These small circular spots appear almost always after a few days of rain; but once started, the transformation of the mycelium in a given area of the leaf is very rapid. In fact, usually a few days suffice for the translucent spot to become thicker than the surrounding portion of the leaf and to assume at first a brownish-red coloration,

which turns almost black at maturation (Plate I; 4). Shortly after the appearance of these spots, the remaining portion of the leaf becomes slightly distorted and assumes a concave-convex appearance at the margin of the developing sclerotia.

The formation of the resting organs in the infected portion of the leaf may occur rapidly if the diseased leaves are placed in vessels with moistened paper in the bottom. The abundant degree of moisture that conditions largely all the other developmental stages, determines also the formation of the sclerotia. These organs develop with difficulty or not at all on the infected leaves of a potted plant kept in a rather dry atmosphere in the laboratory. On the other hand, if the foliage is covered with a bell jar after an abundant watering, the sclerotia usually appear without delay.

The number and the size of the sclerotia vary considerably. It seems that this variation might be ascribed, to a large extent, to the size of the infected portion of the leaf. The following experiment was made to clarify this point. Leaves collected outdoors on June 1, 1936, were placed in moist chambers. Examined each day, these leaves, 35 in number, had produced only two small sclerotia on June 9. This first lot of leaves, with only small infections, hardly produced any sclerotia, probably because the mycelium, which was only slightly developed, had ceased to grow even in the presence of moisture, on account of the room temperature being too high. Another lot of leaves, collected on June 5, gave different results, which are summarized in Table I.

TABLE I

RATE OF FORMATION OF SCLEROTIA ON INFECTED LEAVES

	June 5	June 7	June 8	June 9	June 10
Leaves without sclerotia	90	68	41	24	22
Leaves with sclerotia	0	22	49	66	68

It may be noted in the above table, that 68 out of 90 leaves had produced sclerotia after five days of observation, and 22 failed to do so. This shows that the mycelia in the leaves collected June 5 had developed sufficiently since June 1 to be able to produce a large number of sclerotia even at room temperature. In order to complete the study of the influence of the size of the lesions on the development of the sclerotia, more leaves were submitted to the moist chamber experiment, but this time they were divided into three groups of 25 according to the percentage of infected surface.

Table II, which summarizes the results of this experiment, shows that several sclerotia developed in badly diseased leaves, whereas almost every one of those showing only a portion of discoloured surface produced very few or none. Sclerotia were obtained also from the leaves artificially inoculated in the laboratory, but this occurred only in the case of fairly severe infection.

TABLE II

DEVELOPMENT OF SCLEROTIA ON LEAVES INFECTED TO VARIOUS DEGREES

Percentage of infected surfaces	Number of leaves without sclerotia		
	June 8	June 9	June 10
100	25	5	1
50	25	22	18
25	25	25	25

The development of these organs does not appear to be affected by the drying of the surface of the leaves for a few days after the discoloration of the tissue, provided, however, that the surface be abundantly moistened again.

Fall of the Sclerotia

The fall of the sclerotia usually begins shortly after their formation and maturation. This happened about June 15, in 1936. Usually a fissure appears in the leaf tissue at the margin of the sclerotia, the latter separate and fall, leaving holes in the blade which have exactly their shape; but occasionally the whole leaf falls before the resting organs. At the end of July it is already fairly difficult to find sclerotia on the trees, or if present, the mere touching of the leaves is sufficient to provoke their fall to the ground. In colder regions of Quebec, however, the sclerotia develop and remain on the leaves later in the season. On the ground, the sclerotia await the favourable conditions of the following spring to resume the cycle.

Considerations on the Life Cycle

The life history of this pathogen may be summarized as follows:

1. Hibernation on the ground in the sclerotial condition.
2. Development of apothecia on the sclerotia at the beginning of spring.
3. Ejection and dissemination of the ascospores shortly thereafter.
4. Incubation period varying from 15 days to 3 weeks after inoculation of poplar leaves.
5. Appearance of spots toward the end of spring.
6. Development of sclerotia on poplar leaves at the beginning of summer.
7. Maturation and fall of the sclerotia 15 days or a month later.
8. Resting period extending until the following spring.

In a diagram (Fig. 2), this life cycle is represented in the order of succession of the different stages, and is superimposed on the period of vegetation of the suscept. The diagram is based upon the behaviour of the pathogen and its suscept in 1936, when the epiphytotic was most severe. In other years, perhaps the stages of the cycle will not appear exactly at the same dates and will not necessarily correspond with the same stages of the development of the poplar foliage. As the biological observations of 1936 were made when

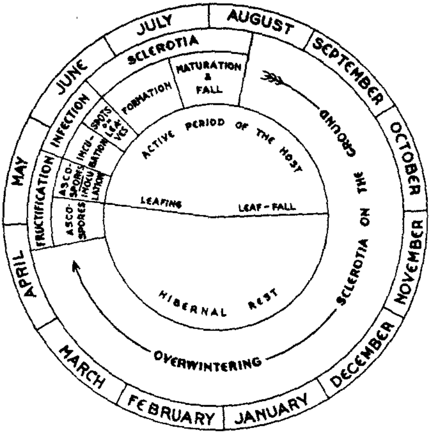

FIG. 2. *Diagram representing the life cycle of Sclerotinia bifrons and the main phases of development of leaves of Populus tremuloides.*

the conditions of infection were ideal, it is considered that the important stages in the development of the parasite and the parasitized coincided perfectly.

On the whole, this organism has a very short life cycle, which includes a single generation of spores to enable the dissemination of the disease. The fruiting period must therefore begin before the foliation of the suscept in order to provide the necessary inoculum for the period of receptivity of the latter, that is, when the young leaves are still sensitive to the infection. If the climatic conditions are favourable at that time, the stages of development will follow each other in the order mentioned above, and will end with the fall of the resting organs and a more or less severe defoliation of the affected trees.

Ecological Observations

Periodically, the disease seems to appear more severely in certain regions. In no place, however, have the reasons of this recrudescence in the strength of the parasitism of the fungus been determined. The epiphytotic of the ink-spot disease of poplar in 1936 enabled the writer to point out the influence of the climatic conditions on the development of the causal agent (Fig. 3).

The present phenological observations, though incomplete and restricted to only one season and to a single locality, have enabled the writer, however, to outline the conditions that may bring about the epiphytotic.

The stages in the life cycle of the parasite, which were recorded in the order of their appearance in 1936, were correlated with the temperature and humidity for the period of infection. In Fig. 3, curves showing weekly means of temperature and air humidity at the Berthierville nursery for the years 1934 to 1937 appear to be fairly significant. In considering the behaviour of each one of these factors during the season of vegetation of 1936, it is noted first that the temperature remained fairly low, with an average of less than 35° F. *until the first few days of May; and second that the moisture of the air during* the first two weeks of May was relatively high, with an average of about 90%. From this it appears that the low temperature in April and the beginning of May favoured the development of the fruit bodies of the parasite, and that the high degree of moisture and the abundant rains facilitated the inoculation of the suscept, which was just in process of foliation in May.

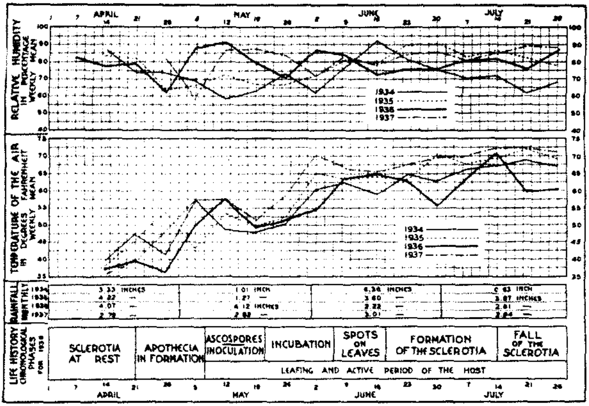

FIG. 3. *Graphic summary of the main phases of the development of the disease in relation to certain climatic factors during the years 1935, 1936, 1937.*

With such a good beginning, the disease could hardly fail to reach an epiphytotic rate. Once started in the leaves, the infection could hardly fail to proceed, especially if favoured by the recurrence in June, as happened in 1936, of damp and relatively cold periods that coincided with appearance of the spots and development of the sclerotia.

A comparison of the curves of the climatic factors for the two years that preceded the epiphytotic with those of the same factors but for the year that followed it shows that it is possible to explain, at least partially, the failure of the disease during these three seasons. It is noted that the temperature rose

much sooner at the beginning of the seasons 1934, 1935, and 1937 than in 1936. Furthermore, and this is perhaps the most important point, the degree of moisture was very low during the period of infection, that is from April 15 to about May 15, thus inhibiting the development of the fruiting organs and consequently the dissemination. If, later, however, a moist atmosphere lasts a few more days, the temperature may then be too high, or the substratum no longer in a state of receptivity, as the cuticle of the leaves may have hardened.

In 1934, the attacks of the fungus on the foliage were very weak. The following year, a notable proportion of the leaves, especially in shaded places on the border of the wood, was infected. An epiphytotic followed in 1936 to decrease to almost nothing in the spring of 1937. The extent of the disease was very severe in 1936, mostly on account of eminently favourable conditions, but the abundance of the inoculum had also something to do with it. In fact, the infection of 1935, though fairly weak, did nevertheless contribute to a general infestation and supplied the inoculum for the following year. The writer is convinced that the climatic factors control largely the development of the parasite, but the occurrence of a fairly important centre of infection in a stand is also a factor that should not be neglected.

Another condition essential to the occurrence of an outbreak is a fairly dense stand of young poplars with the foliage not too far from the soil. Although without accurate measurements on the influence of the density, purity, or age of a population upon the degree of infection, the disease was observed to be especially severe in places where a large number of young poplars were growing. It is well known that this type of stand occurs frequently in the Province of Quebec in natural reforestation a few years after the disappearance of the first stand by cutting or fire, or the abandonment of cultivated lands. The young birches or aspens then form a thicket during the period preceding the large sapling stage.

The author's observations were made chiefly in a stand that consisted almost exclusively of poplars mixed now and then with birches. Consequently, with the climatic conditions which prevailed in 1936 and abundant inoculum on the ground, the outbreak could not fail to be severe at the border of an old wood. This was as serious, if not more so, as any in all the regions that were visited in the Province of Quebec and also in numerous places reported to the writer. The pure young stands, however, were those especially affected.

The set of conditions essential to an epiphytotic development of the parasite appears to be that mentioned above, for the disease remains mild or disappears if a definite factor fails in the environment. Such was the case in 1937. In the same plot that was badly affected the previous year, very few leaves were infected, in spite of the abundant inoculum on the ground. This decrease may be easily accounted for by the rapid rise in temperature in the middle of April, followed by a dry period.

It could be objected to this explanation that the failure of fruiting of the very abundant sclerotia resulted from the unfavourable conditions of the winter or from any other unknown cause. It could be answered that these

organs of preservation had all the necessary vitality, for they produced fruit bodies in a refrigerator and even outdoors on a protected cold soil.

On the whole, the conditions favouring the epiphytotic development of the disease are the following: (i) Presence of a sufficient inoculum: sclerotia in fairly large numbers on the soil; (ii) Occurrence of a dense stand of young poplars; (iii) Fairly low temperature before or at the time of foliation; (iv) Fairly high degree of moisture during foliation.

Pathological Observations

In the course of this study, observations were made as to the effects of the disease on the suscept. Material was collected and prepared for histological study of the diseased tissues of the leaf, but this still fragmentary work will not be considered here. The following considerations deal rather with the effects of the infection on the tree as a whole and therefore on the forest population.

In 1935, the limits of a sample plot were marked in the young stand which served for making almost all the above observations. All the trees in the plot were numbered and an enumeration, for each tree, of healthy and diseased leaves was effected before the fall of the latter. Taking into account the dead trees also, this count was repeated in 1936 on the same individuals. The results are summarized in Table III.

TABLE III

NUMBERS OF LEAVES INFECTED BY *Sclerotinia bifrons* IN A SAMPLE PLOT OF *Populus tremuloides*

	1935			1936		
	Total number	Average per tree	%	Total number	Average per tree	%
Trees	74			74 (11 dead)		
Leaves	31,186	421.4		21,338	288.3	
Healthy	30,195	408.1	96.9	2,172	29.3	10.2
Diseased	991	13.3	3.1	19,166	259.0	89.8

It may be seen at once in the above table that the infection was considerably more severe in 1936 than in 1935. In spite of the small percentage of disease in 1935, 11 trees died as a result of a total defoliation or a partial reduction in the leaf surface. This infection had additional effects in reducing considerably the vitality of the trees that remained alive. So instead of an increase in the number of leaves in 1936, as should normally occur after a year of growth, a decrease was recorded.

Unfortunately these counts were not repeated in 1937 to show the effects of the previous infection, which was so much more severe than in 1935. How-

ever, a superficial inspection of the trees showed that a large number of them were dead, the foliage was pale, and the shoots were short and few. The mortality was high especially among the young trees. The taller individuals, from 8 to 12 ft., though severely affected, were not killed because a fairly large proportion of the uppermost leaves remained healthy in 1935.

On the whole, the small trees may be killed by a severe infection in the course of an outbreak, but the taller individuals are rarely affected to such an extent as to die. From the standpoint of the tree population, the damage cannot be very serious even in the case of a widespread epiphytotic, for the smaller trees are frequently doomed to disappear sooner or later anyhow, being suppressed by the stronger and taller individuals, which recover with the return of normal conditions. However, in certain stands or plantations to be used for special purposes, such as wood for matches, the losses may be fairly heavy.

Conclusions

The results of investigations and observations on a disease that appears to be important in certain years are presented as a contribution to the study of the causal fungus under all its aspects, practical as well as scientific.

This organism is, according to all evidence, a North American endemic, with a geographical range that appears to coincide somewhat with that of *Populus tremuloides*, its chief suscept. It was found also on other American species of the same genus, and on the Lombardy poplar, but always within the range of the trembling aspen.

The identity of the parasite is now well established and its perfect stage has recently been properly named. Whetzel was the first to find the fructification of this fungus and to separate it from certain associated saprophytic species.

The following conclusion should be drawn from the results of the biological and ecological observations presented above: this fungus, which produces annually a single generation of spores and requires fairly favourable conditions for its development, cannot be considered a very important destructive agent of trees and tree populations. It usually happens that one or other of the conditions essential to infection fails to appear simultaneously with the others and at the proper moment. It is possible, however, that young stands of poplars, which have a greater value than the thicket representing the first step of reforestation, may be more severely affected by this disease, either because the environment might be very favourable to the development of the pathogen, or because susceptible species, strains, or varieties might have been introduced or developed.

As a final consideration, it may be added that this American indigenous pathogen has rather mild effects under normal conditions. It might be quite different with a parasite of the same group but of foreign origin, because this might assume the highest degree of virulence on one or several of our forest species, even under the usual conditions.

Acknowledgments

The writer wishes to express his appreciation to Dr. H. H. Whetzel, Professor of Plant Pathology at Cornell University, for his valuable suggestions and criticisms in revising the manuscript. The writer is also indebted to Mr. Emile Jacques, graduate student at Cornell University, who has translated this paper originally written in French.

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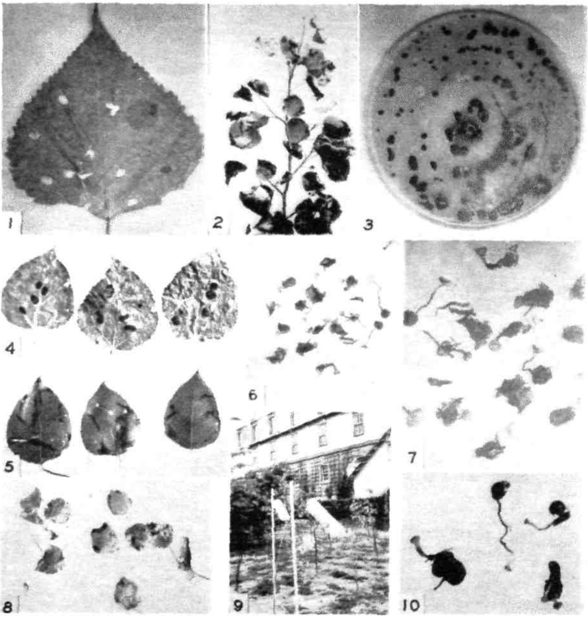

PLATE I. 1. *Leaf of Populus canadensis infected with Sclerotinia bifrons, showing sclerotia and holes left by the fall of other sclerotia* (× ½). 2. *Twig of P. tremuloides with leaves all affected by the disease* (× ¼). 3. *Sclerotia of S. pseudo-bifrons produced abundantly on potato-agar in Petri dish* (× ½). 4. *Dead leaves just before the fall of sclerotia* (× ½). 5. *Infected leaves, showing linear spots* (× ½). 6. *Sclerotia bearing one to three apothecia* (× ⅓). 7. *Matured apothecia of S. bifrons* (× ⅔). 8. *First signs of the disease on leaves of P. tremuloides* (× ¼). 9. *Celluloid inoculation chambers used on outside trees.* 10. *Different stages of development of apothecia* (× 1).

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EFFECTS OF TWO PREPARATIONS OF NAPHTHYLACETIC ACID ON THE GERMINATION AND EARLY GROWTH OF WHEAT SEED DAMAGED BY FORMALDEHYDE[1]

BY N. H. GRACE[2]

Abstract

Two varieties of wheat seed were sprinkled with solutions containing formaldehyde and naphthylacetic acid to give treatments of 1 and 10 parts of the latter to a million parts of seed by weight. In both varieties formaldehyde treatment reduced the germination rate, final germination count, and the air-dry weight of stems and roots at 29 days after planting. Some reduction in root suppression resulted from the 1 p.p.m. concentration of hormone; the higher level had no effect.

The experiment involved the use of two preparations of naphthylacetic acid, differing only in respect to a trace of halogen in one. This impurity had no effect on final germination or stem and root weights, but increased the germination rate. The trace of halogen had the more marked effect at the highest concentration and the variety Marquis was affected to a greater extent than Reward.

A recent communication reported an experiment in which wheat seed was soaked in solutions of formaldehyde and naphthylacetic acid (3). While the results from blotting paper germination indicated some reduction in formaldehyde injury on treatment with naphthylacetic acid, germination and early growth in soil failed to show any reduction in the injury. The present communication describes an experiment in which seed wheat was treated by a sprinkling technique, a method that permits application of a definite weight of plant hormone chemical to a given weight of seed. Further, two preparations of 1-naphthylacetic acid, known to differ slightly, were used. In this manner, additional information was obtained on the response of formaldehyde treated seed to definite amounts of naphthylacetic acid, and the possible significance of traces of other chemicals in the hormone were investigated.

Experimental

Naphthylacetic acid solutions containing 5 and 50 p.p.m. were prepared in a 1 : 320 solution of commercial formaldehyde (37% by weight of the gas). Two different preparations (2, 5) of naphthylacetic acid were used in the investigation, differing only in respect of a trace of halogen shown to be present in one by means of a flame test.

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. N.R.C. No. 908.
[2] *Biochemist.*

Two varieties of wheat, Marquis and Reward, were used in an effort to determine whether there was any difference in the response of varieties to either concentration or preparation of naphthylacetic acid. Solutions were applied by the sprinkling method of treatment, 10 cc. to 50 gm. of seed, effecting treatments of 1 and 10 p.p.m. (parts of naphthylacetic acid to one million parts of wheat by weight). The treated seed was held in the manner previously described (3), and planted in sterilized soil in units of 50 seeds in small cardboard flats, approximately 24 hr. after treatment. These were kept in a greenhouse room which ranged in temperature from 60 to 68° F.

The experiment comprised four treatments consisting of an untreated control, a formaldehyde control, and the two treatments with formaldehyde and naphthylacetic acid at levels of 1 and 10 p.p.m. The two varieties were placed at random in duplicate sub-blocks, each a Latin square, within a large block. Treatments were randomized within sub-blocks in the form of double plots (two flats) containing the two different naphthylacetic acid preparations. There were also double plots of the untreated and formaldehyde treated controls in order to give the averages of hormone concentrations and controls the same weight. Each sub-block contained eight replicates of the treatments, or four replicates of the naphthylacetic acid preparations; the entire experiment involved the use of 128 flats. This arrangement provided three levels of precision for the various comparisons; these were, in descending order, between preparations, treatments, and finally varieties.

Seed was planted on March 17, 1939, and daily germination counts were made as soon as plants emerged; counts were continued until germination was virtually complete. The daily counts were used to compute germination rates by the method of Bartlett (1). The plants were washed out of soil 29 days after planting, placed in an oven at 95° C. for two hours, conditioned in the laboratory for a week, and air-dry stem and root weights determined (4). All data on germination rates, final germination counts, and air-dry weights were subjected to analyses of variance.

Results

In Table I are given data from the analyses of variance. There were significant treatment effects with respect to all four characters considered. The germination rates also showed significant differences between the two preparations of naphthylacetic acid, and the interactions between preparations and dosage and preparations and varieties also were significant.

The average germination rates, final germination count, and air-dry weight of stems and roots are given in Table II. In each case formaldehyde alone effected injury. While 1 p.p.m. of naphthylacetic acid did not increase final germination significantly above the formaldehyde control, the value obtained was not significantly below the untreated control. All treatments gave air-dry weights of stems lower than that of the untreated control; and weights with both hormone concentrations failed to differ from the formaldehyde control. While all formaldehyde treatments were below the untreated control

TABLE I

ANALYSIS OF VARIANCE OF RESPONSE OF WHEAT SEED TREATED WITH FORMALDEHYDE AND NAPHTHYLACETIC ACIDS

Source of variance	D.f.	Mean square			
		Germination rate	Final germination count	Weight of stems	Weight of roots
Blocks between varieties	1	.0795	24.50	.1152	.00173
Within varieties (error (a))	2	.2661	102.58	.0554	.00886
Rows	12	.0291***	89.13*	.0229*	.00344**
Columns	12	.0164**	29.28	.0193*	.00414**
Treatments	3	.0198*	175.55*	.1093***	.05268***
Treatments × squares between varieties	3	.0031	7.69	.0070	.00134
Error (b)	30	.0058	42.10	.0086	.00106
Between preparations	1	.0418**	1.12	.0001	.00080
Preparations × dosage	1	.0215*	36.12	.0030	.00001
Preparations × varieties	2	.0217**	60.62	.0001	.00462
Error (c)	28	.0047	53.55	.0141	.00163

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

TABLE II

AVERAGE EFFECTS OF FORMALDEHYDE-NAPHTHYLACETIC ACID TREATMENTS ON GERMINATION AND EARLY GROWTH OF WHEAT SEED IN SOIL (MEAN OF TWO VARIETIES)

Treatments	Germination rate	Final germination count, %	Air-dry weight of stems	Air-dry weight of roots
Untreated	.613	41.1	.736	.264
Formaldehyde alone	.572	36.9	.601	.172
With 1 p.p.m. naphthylacetic acid	.608	38.5	.645	.194
With 10 p.p.m. naphthylacetic acid	.564	35.7	.628	.188
Necessary difference, 5% level	.038	3.3	.047	.017

in respect of air-dry weight of roots, both hormone concentrations were above the formaldehyde control, the 1 p.p.m. level to a significant extent.

The average germination rate of both varieties was greater by 0.036 after treatment with the naphthylacetic acid preparation containing a trace of halogen. This difference was more marked at the greater hormone concentration, and was 0.010 at the 1 p.p.m. level and 0.062 at the 10 p.p.m. The varieties reacted somewhat differently to the two preparations, the presence of halogen increasing the average germination rate of Reward by 0.029 and Marquis by 0.043. This varietal effect was in evidence at both concentrations of phytohormone.

Some reduction of formaldehyde injury is indicated at the 1 p.p.m. level of treatment, a result in agreement with earlier findings in which 1 p.p.m.

indolylacetic acid in formaldehyde increased the air-dry weight of stems of plants grown in soil (3). It is interesting to note that traces of halogen impurity failed to affect final germination or air-dry weight of stems and roots but did increase the germination rate. It may be concluded that minor impurities in growth stimulating chemicals can have a significant effect on growth responses.

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VARIETAL DIFFERENCES IN BARLEYS AND MALTS

X. CORRELATIONS OF CARBOHYDRATES WITH NITROGEN FRACTIONS AND WITH MALT EXTRACT, STEEPING TIME, AND MALTING LOSS[1]

By Henry R. Sallans[2] and J. Ansel Anderson[3]

Abstract

Glutelin is the only nitrogen fraction that is significantly correlated with starch, barley extract, and insoluble carbohydrate, between varieties. In each case the correlation coefficient barely attains the 5% level of significance. Within varieties the correlation coefficients for starch and barley extract with glutelin, hordein, and salt-soluble nitrogen are all negative and attain the 1% level of significance. Insoluble carbohydrate shows no intra-varietal associations with any of the nitrogen fractions.

Starch and barley extract are very closely associated with malt extract both within and between varieties. Insoluble carbohydrate is closely related to malt extract between but not within varieties. It is shown that Bishop's principle of regularities in the carbohydrate and nitrogen composition within varieties fails to apply to insoluble carbohydrate. Intra-varietal associations of steeping time with starch, $r = 0.797$, barley extract, $r = 0.730$, and insoluble carbohydrate, $r = -0.782$, are not dependent on the total nitrogen of the barleys.

Regression coefficients of malt extract on barley extract are homogeneous both within and between varieties, and the average varietal and station regressions do not differ significantly. It is shown that barley extract is more closely related to malt extract than either starch or insoluble carbohydrate, between varieties. Within varieties it affords a more accurate estimate of malt extract than either starch or total nitrogen.

Malt extract is composed mainly of the carbohydrate and nitrogenous materials of the barley from which the malt is made. Therefore it appears logical that any successful prediction of malt extract should take cognizance of both these important constituents of the barley. In pursuing the search for relations on which to base such a prediction, the correlations of the carbohydrate fractions of barley with barley nitrogen fractions and with malt extract, steeping time, and malting loss have been investigated. The results of this study are discussed in the present paper.

Part IX (2) of this series of papers dealt with varietal differences in the carbohydrate composition of barleys and their relations with total nitrogen and 1000-kernel weight. In view of the importance of relations between nitrogen and carbohydrate, it appeared advisable to investigate these in greater detail. The present study was, therefore, extended to include correlations with insoluble nitrogen, alcohol-soluble nitrogen, and total salt-soluble nitrogen.

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Data

Experimental data were obtained from 144 samples of barley representing 12 varieties grown at 12 widely separated experimental stations in Canada. Malting methods and summaries of the data on malt extract, malting loss, and steeping time were given in Part IV (6). The data on total, insoluble, alcohol-soluble, and salt-soluble nitrogen were presented in Part I (1) together with a brief description of the barley varieties and the conditions under which they were grown. The methods used and the results obtained for the carbohydrate fractions of barley appeared in Part IX (2).

Correlations between Carbohydrate and Nitrogen Fractions of Barley

The simple inter-varietal and inter-station correlation coefficients between the carbohydrate and nitrogen fractions of barley are shown in the upper half of Table I. The corresponding partial correlation coefficients, independent of total nitrogen, are given in the bottom half of the table. Following the plan of other papers (7, 8) in this series, it seems advisable to discuss the inter-varietal and inter-station relations in separate sections.

TABLE I

SIMPLE AND PARTIAL CORRELATION COEFFICIENTS, INDEPENDENT OF TOTAL NITROGEN, BETWEEN CARBOHYDRATES AND NITROGEN FRACTIONS OF BARLEY

Barley property	Inter-varietal				Inter-station			
	Total nitrogen	Insoluble nitrogen	Alcohol-soluble nitrogen	Salt-soluble nitrogen	Total nitrogen	Insoluble nitrogen	Alcohol-soluble nitrogen	Salt-soluble nitrogen
Simple								
Starch	−.399	−.582*	−.060	.018	−.953**	−.890**	−.939**	−.801**
Barley extract	−.401	−.590*	−.115	.109	−.908**	−.857**	−.897**	−.744**
Nitrogen in extract	.241	−.387	.077	.821**	.924**	.807**	.875**	.966**
Cellulose-lignin	.293	.634*	.012	−.212	−.320	−.188	−.298	−.482
Partial								
Starch	—	−.511	.490	.126	—	−.062	−.004	.065
Barley extract	—	−.519	−.392	.229	—	−.102	−.036	.149
Nitrogen in extract	—	−.330	−.209	.810**	—	.336	−.535	.892**
Cellulose-lignin	—	.591	−.403	−.303	—	.304	.108	−.422

*In this and later tables, ** denotes that the 1% level, and * that the 5% level of significance is attained.*

Inter-varietal Relations

In general, the inter-varietal simple and partial correlation coefficients are very similar in magnitude. The simple coefficients for the correlation of insoluble nitrogen with starch, extract, and cellulose-lignin residue are sig-

nificant, but the partial coefficients just fail to attain the 5% level. However, it is obvious that these simple and partial coefficients do not differ significantly, and hence the effects of total nitrogen are not sufficient to explain the simple correlations between varietal means.

In Part IX (2) it was shown that barleys having a low kernel weight tend to be low in the reserve carbohydrates, starch and barley extract, and high in the structural materials, cellulose and lignin. The correlation coefficients of insoluble nitrogen (structural protein (3)) with starch, extract, and cellulose-lignin residue are similar in magnitude and sign to those obtained with 1000-kernel weight. It appears that there is an association between structural carbohydrates and structural proteins, and as the proportion of these increases there is a compensating decrease in the proportion of reserve carbohydrates. Furthermore, these relations are associated to some extent with the kernel weight of the barley. Apart from these rather loose associations between the carbohydrates and structural protein, there appears to be no other inter-varietal relation between the carbohydrates and the nitrogen fractions of the barleys.

A fairly close relation exists between the nitrogen in the barley extract and total salt-soluble nitrogen. It seems that the barley wort acts in much the same manner as a dilute salt solution, with the result that it contains essentially the same nitrogen fractions as the 5% potassium sulphate extract.

Inter-station Relations

The simple inter-station correlation coefficients in the upper right corner of Table I show that starch and barley extract are negatively associated with all the barley nitrogen fractions and that extract nitrogen is positively associated with them. Since total nitrogen is closely related to these nitrogen fractions (1, 3) and also to starch, extract, and extract nitrogen, partial correlation coefficients, independent of total nitrogen, were computed to eliminate these common associations. These partial coefficients, shown in the lower right corner of the table, all fall below the 5% level of significance with a single exception, i.e., that between wort nitrogen and salt-soluble nitrogen. This exception results from the fact that barley extract nitrogen is essentially the same fraction as salt-soluble nitrogen. It will be observed that the simple inter-station correlation coefficient between extract nitrogen and salt-soluble nitrogen is 0.966, while that between salt-soluble nitrogen and total nitrogen (1) is 0.851. Thus extract nitrogen is more closely related to salt-soluble nitrogen than the latter is to total nitrogen. This results in a significant partial correlation independent of total nitrogen.

From these correlation studies, it is evident that, within a given variety grown at a number of stations, total nitrogen content is associated with all the nitrogen fractions determined (1) and also with the carbohydrate and nitrogen fractions that contribute to barley extract. The only property measured that does not appear to follow this principle of regularity first enunciated by Bishop (3, 5) is the insoluble cellulose-lignin residue. It is

interesting to note that while the data are not given, the nitrogen content of this residue showed significant correlations with the various nitrogen fractions and that these relations were dependent on common associations with total nitrogen. Thus the only exception to the principle of regularity of composition within varieties appears to be with respect to the structural carbohydrate fraction. Bishop's data (4) also show this same exception. There appears to be no satisfactory explanation of this phenomenon, but it is interesting that the partial correlation studies in Part IX (2) indicate that the structural residue is negatively associated with both starch and barley extract when the effects of total nitrogen are removed. Thus this fraction is anomalous in that it shows regularities with the carbohydrate fractions but not with the nitrogen fractions.

Correlations between Carbohydrate Fractions of Barley and Malting Properties

The simple inter-varietal and inter-station correlation coefficients between the carbohydrate fractions of barley and malt extract, wort nitrogen, steeping time, and malting loss are shown in the upper half of Table II. The corresponding partial correlation coefficients, independent of total nitrogen, are given in the lower half of the table.

TABLE II

SIMPLE AND PARTIAL CORRELATION COEFFICIENTS, INDEPENDENT OF TOTAL NITROGEN, BETWEEN CARBOHYDRATES OF BARLEY AND MALTING PROPERTIES

Barley property	Inter-varietal				Inter-station			
	Malt extract	Wort nitrogen	Steeping time	Malting loss	Malt extract	Wort nitrogen	Steeping time	Malting loss
Simple								
Starch	.854**	−.060	−.174	.359	.967**	−.843**	.793**	.359
Barley extract	.914**	.074	−.311	.428	.973**	−.748**	.814**	.428
Nitrogen in extract	.642*	.833**	−.717**	.856**	−.831**	.672*	−.520	.856**
Cellulose-lignin	−.901**	−.115	.284	−.475	.142	.089	−.367	−.356
Partial								
Starch	.828**	.007	−.089	.374	.625*	−.587	.797**	.218
Barley extract	.899**	.155	−.245	.499	.854**	−.203	.730**	.158
Nitrogen in extract	.617*	.828**	−.700*	.839**	.475	−.138	.233	.247
Cellulose-lignin	−.893**	−.173	.230	−.485	−.596	.546	−.782**	−.195

Inter-varietal Relations

The inter-varietal simple and partial correlation coefficients are of the same order of magnitude, which indicates that the relations depicted in the table are not dependent on relations with total nitrogen. This result was expected since it has been shown in previous studies that the nitrogen content of the

barleys showed no inter-varietal associations with other barley and malt properties. A single exception to this generalization is a significant association between total nitrogen content and the reserve protein hordein, $r = 0.811^{**}$ (1).

It appears from the correlation coefficients between malt extract and the properties listed in the first column of Table II, that the regularities in the carbohydrate constituents of barley (2) are definitely related to the yield of malt extract. The coefficients for starch and barley extract are highly significant and positive, while that for the cellulose-lignin fraction is highly significant and negative.

The only other significant inter-varietal correlation coefficients are those between extract nitrogen and wort nitrogen, steeping time, and malting loss. It appears that these coefficients are significant because extract nitrogen is related to salt-soluble nitrogen and, if partial correlations are computed, independent of this latter property, they all fall below the 5% level of significance.

Inter-station Relations

Within varieties starch and barley extract are positively related to malt extract; extract nitrogen is negatively related; and the cellulose-lignin residue shows no association with malt extract. As would be expected, these relations conform to the regularity principle which exists within varieties. However, it is again evident that the cellulose-lignin residue fails to fit into the regularity. The partial correlation coefficients, independent of total nitrogen, of malt extract with starch and barley extract are significant. This indicates that the regularity principle does not hold rigidly for these relations, since starch and barley extract are more closely related to malt extract than is total nitrogen. It appears that total nitrogen reflects the total carbohydrate of the barley or malt, by difference, but since it fails to reflect the insoluble structural carbohydrate, it is not as closely related to malt extract as direct measures of the reserve carbohydrates.

The significant correlations of wort nitrogen with starch, barley extract, and extract nitrogen reflect the intra-varietal regularities between nitrogen and carbohydrate. The partial coefficients, independent of total nitrogen, again serve to clarify these relations.

Steeping time is positively associated with starch and barley extract and these relations are not dependent on total nitrogen, as shown by the significant partial correlation coefficients. The insoluble residue (cellulose-lignin) is negatively associated with steeping time when the masking effects of total nitrogen are removed by computing the partial coefficient. An increase in the reserve carbohydrates, which contribute to malt extract, results in a longer steeping time or a slower rate of water absorption in the steep. Conversely, an increase in the cellular or structural material is accompanied by a decrease in steeping time or a more rapid absorption. It can be shown that these relations are not entirely dependent on 1000-kernel weight, since

the partial correlation coefficient, independent of 1000-kernel weight, between starch and steeping time just fails to attain the 5% level, while that for barley extract just exceeds it.

Malting loss is positively correlated with extract nitrogen, but the partial coefficient, independent of total nitrogen, indicates that this is merely the reflection of a more fundamental association with total nitrogen.

Regression of Malt Extract on Carbohydrate Fractions of Barley

The correlation coefficients presented in the preceding sections indicate that: starch, barley extract, and cellulose-lignin residue appear to be useful factors for the prediction of malt extract from different varieties grown at the same station; and, starch, barley extract, and total nitrogen appear to be the most suitable factors for prediction within a given variety grown at different stations. These coefficients were computed from varietal and station means, and while they serve to indicate whether the properties are associated with malt extract, they fail to furnish information as to whether these associations are equally close for each variety and each station. To obtain a better understanding of these relations, regressions by stations and varieties were determined and tested for homogeneity. Analyses of residual variance for inter-station or varietal regressions of malt extract on total nitrogen, starch, and barley extract are given in Table III; corresponding analyses for inter-varietal or station regressions on cellulose-lignin residue, starch, and barley extract, appear in Table IV.

TABLE III

ANALYSES OF RESIDUAL VARIANCE FOR VARIETAL REGRESSIONS OF MALT EXTRACT ON TOTAL NITROGEN, STARCH AND BARLEY EXTRACT

Variance due to	D.f.	Mean square		
		Total nitrogen	Starch	Barley extract
Differences among varietal regression coefficients	11	0.3333	0.4275	0.4641
Deviations of varietal means from average regression	11	40.9373**	12.8655**	7.7271**
Residual deviations from individual varietal regressions	120	0.8085	0.6073	0.5589
Deviations from average varietal regression	131	0.7406	0.5998	0.5510

** *Exceeds mean square residual, 1% level of significance.*

To illustrate the discussion and to provide a clearer interpretation of the statistical analyses, the relations between malt extract and barley extract are presented graphically. The scatter diagram, Fig. 1, shows the complete data for these properties, while in Fig. 2 the relation is broken down into its inter-varietal and inter-station components.

The analyses of residual variance, Table III, indicate that the regression coefficients of malt extract on barley extract within varieties do not differ significantly. This means that these regressions, shown in the small scatter

TABLE IV

ANALYSES OF RESIDUAL VARIANCE FOR STATION REGRESSIONS OF MALT EXTRACT ON CELLULOSE-LIGNIN RESIDUE, STARCH AND BARLEY EXTRACT

Variance due to	D.f.	Mean square		
		Cellulose-lignin residue	Starch	Barley extract
Differences among station regression coefficients	11	0.5982	0.9318	0.3408
Deviations of station means from average regression	11	66.8654**	3.5033**	2.9652**
Residual deviations from individual station regressions	120	1.5436	1.4276	1.0378
Deviations from average station regression	131	1.4642	1.3859	0.9507

** *Exceeds mean square residual, 1% level of significance.*

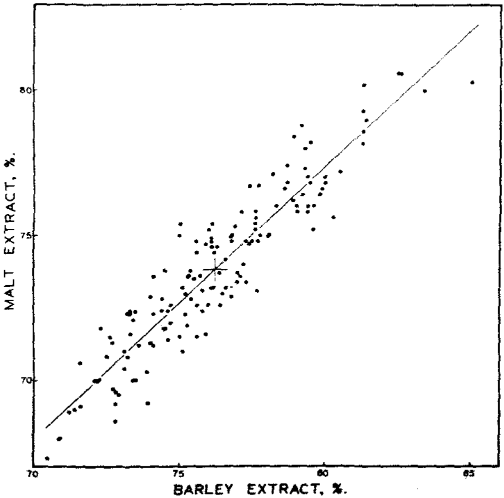

FIG. 1. *Scatter diagram showing the relation between barley and malt extracts.*

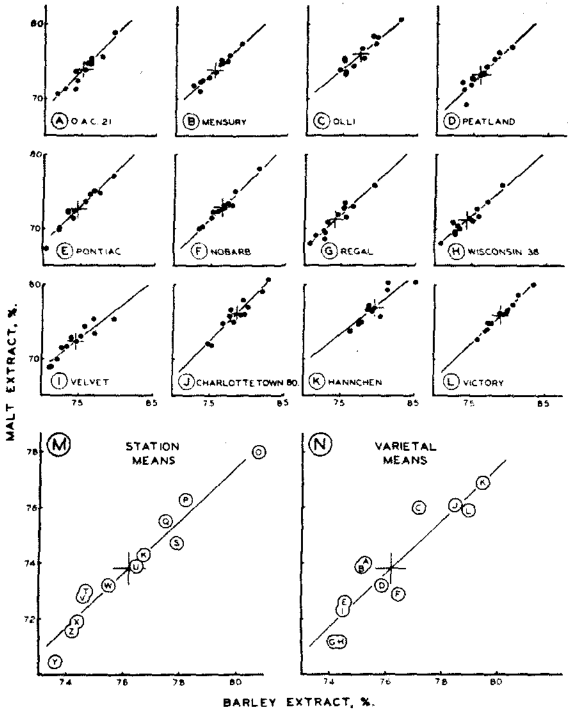

FIG. 2. *Scatter diagrams showing varietal and station relations of barley and malt extracts. The key to stations follows: O, Nappan; P, Fredericton; Q, Ste. Anne de Bellevue; R, Ste. Anne de la Pocatière; S, Lethbridge; T, Winnipeg, U, Brandon; V, Guelph; W, Ottawa; X, Lacombe; Y, Beaverlodge; Z, Gilbert Plains. The key to varieties is given in the small diagrams A to L.*

diagrams *A* to *L* (Fig. 2), may be regarded as a series of parallel lines having the same slope. However, reference to Table III shows that the centroids, which represent varietal means for the properties, deviate significantly from the average varietal regression. If the centroids, marked with a cross in the small diagrams, are superimposed on the average varietal regression, shown in the lower right diagram *N* in Fig. 2, the deviations from the regression line are significant. The analyses of variance indicate that the relations of

malt extract to starch are very similar to those for barley extract. The regressions for nitrogen may also be represented by a series of parallel lines but in this case the slope is negative. The mean square deviation of the individual values from the average regression, shown in the last line of Table III, indicates that malt extract is more closely related to barley extract and starch than to total nitrogen. By means of an "F" test it can be shown that the mean square residual for barley extract is significantly lower than that for total nitrogen.

The analyses of residual variance for the inter-varietal regressions of malt extract on barley extract, Table IV, show that these regression coefficients do not differ significantly from station to station. A series of small scatter diagrams for stations would be very similar to those for varieties illustrated in Fig. 2 and the regression lines would all have the same slope. The centroids, or station means, are shown in the lower graph M, Fig. 2. From the graph and the analyses in Table IV, it is evident that these centroids deviate significantly from the average station regression. A similar treatment of the data for regression of malt extract on cellulose-lignin residue and starch (Table IV) shows that in each case the regressions can be represented by a series of parallel lines. Those for starch have a positive slope while those for the residue have a negative slope. An "F" test applied to the mean square deviations from the average varietal regressions, shown in the last line of Table IV, shows that the relation of malt extract to barley extract is significantly closer than the relation of malt extract to starch or cellulose-lignin residue.

From the preceding discussion it is clear that both within and between varieties the regressions of malt extract on barley extract give the best fit both for the individual values and for variety and station means. Furthermore, the regression coefficients within and between varieties, 0.982 and 0.948 respectively, do not differ significantly and are very close to the total regression coefficient 0.940. These considerations suggest that a fundamental relation exists between barley and malt extract which may be of value for prediction purposes.

Applications to the Prediction of Malt Extract

The relation between barley and malt extracts, Fig. 1, is sufficiently close to suggest that a useful prediction equation might be derived from it. Since this is a linear relation, such an equation would take the form, "$y = a + bx$", when "y" = malt extract, "x" = barley extract, "b" = the regression coefficient, and "a" = the intercept of the regression line and the major ordinate. In brief, the prediction equation becomes the equation of the regression line shown in Fig. 1 and the standard error of prediction is represented by the standard deviation of the individual observations from this line. If the necessary calculations are made, it turns out that the standard error of prediction is $\pm 1.0\%$ in malt extract and the equation is:

$$y = 2.2 + 0.94x.$$

At first sight this equation, based on barley extract alone, does not appear to be quite as accurate as Bishop's "restricted general equation" (5) [$E = 134.7 - 9.0\,N - 2.8I$, in which E = malt extract, N = total nitrogen, and I = insoluble residue]. Both equations have a standard error of ± 1.0, but in Bishop's equation E is given in brewer's pounds per quarter and represents values lying between 91 and 102, whereas in our equation E is given in per cent Plato and represents values lying between 68 and 80. However, since our data show that between varieties the relation of malt extract to barley extract is closer than that of malt extract to insoluble carbohydrate, and that within varieties the relation of malt extract to barley extract is closer than that of malt extract to total nitrogen, the prediction equation based on barley extract alone will give better results with our barleys than an equation similar to Bishop's.

The general equation for the prediction of malt extract from barley extract can be broken down into a number of individual equations, one for each variety and one for each station. These will be the equations for a series of parallel lines passing through the various centroids (i.e., the points shown in Fig. 2, M and N). Since the centroids do not fall on the major regression line, it is evident that the values of the intercept on the major ordinate will differ. Hence a series of varietal and station equations, having the same values of "b" but differing in the constants "a", can be obtained. These equations would serve to define malt extract more accurately but would be of limited usefulness since they apply only to specific varieties or stations. There appears to be no particular point in listing all these equations. However, for comparison with the general equation and since O.A.C. 21 is the recognized standard of malting quality in Canada, the equation for this variety is given below:

$$y = 3.2 + 0.94x,$$

where y = malt extract and x = barley extract.

It is obvious from the preceding sections that it is possible to derive inter-varietal equations from the regression of malt extract on cellulose-lignin residue or starch, and to derive inter-station equations from the regression of malt extract on total nitrogen or starch. However, since these equations will be less accurate than the corresponding equations based on barley extract, it does not appear worth while to consider them in detail.

The relations of barley and malt extract presented in this and earlier papers indicate that barley extract may well represent the total potential malt extract. However, since there is a lack of complete correspondence between these properties as indicated by varietal and station differences in the values of the constant "a", other factors must operate to determine whether the potential extract is realized. Since the enzymes developed during malting serve to liberate the potentially soluble material during mashing, it seems evident that varietal and station differences in enzymatic activity (7) must contribute to the observed differences in the constants "a". This hypothesis can be tested roughly by means of the data shown in Fig. 2 N and data on

the diastatic activity of the varieties, given in Part IV (3). Those varieties (O.A.C. 21, Mensury, and Olli) that give large amounts of malt extract by comparison with barley extract (points *A*, *B*, and *C*, above the line in Fig. 2*N*) should be high in enzymatic activity; whereas those varieties (Nobarb, Regal, and Wisconsin) that give low amounts of malt extract by comparison with barley extract (points *F*, *G*, and *H*, below the line in Fig. 2*N*) should be low in enzymatic activity. In general this proves to be true. The varieties O.A.C. 21, Mensury, and Olli have diastatic activities of 127, 129, and 153, whereas for the varieties Nobarb, Regal, and Wisconsin the values are 100, 85, and 96 respectively. Similar relations hold for proteolytic and starch liquefying activity and furnish additional support for the hypothesis.

If barley extract is considered as potential malt extract, its determination should be of use to plant breeders in selecting varieties that show promise of having superior malting qualities from a number of hybrid lines of equal agronomic quality. It is evident that, unless a new variety has a barley extract as high or higher than that of the standard malting variety there is little or no chance of its malt extract yield exceeding that of the standard. However, it should be noted that if lines are selected on this basis, some of them may not produce malts of high extract yield because of deficiencies in enzymatic activities. Thus it will only be profitable to apply a prediction based on barley extract to hybrids resulting from a cross in which at least one of the parents is known to be of reasonably high enzymatic activity. Moreover, owing to the differential effect of environment on varieties, if the hybrid material is grown at one station only, local conditions may tend to conceal average varietal differences in extract yield which might become evident if the lines were grown at a number of stations. In spite of these limitations, this method of selection appears to have a definite value. Determinations of barley extract can be made quite rapidly (12 per day per man) and require only a small amount of grain. It should therefore be possible to apply it on a much wider scale and at an earlier stage in the process of selection than is possible with laboratory malting test.

The foregoing discussion serves to illustrate the development and possible utility of prediction equations based on the relations between malt extract and individual barley properties. It is also possible to derive equations involving the simultaneous relations of malt extract and two or more barley properties (e.g., 3, 4). Equations of this type, which permit more accurate prediction, will be discussed in the next paper in this series.

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HYBRIDIZATION OF *TRITICUM* AND *AGROPYRON*

VI. INDUCED FERTILITY IN VERNAL EMMER × *A. GLAUCUM*[1]

By F. H. Peto[2] and J. W. Boyes[2]

Abstract

Colchicine treatments of the seed of a sterile F_1 hybrid of Vernal emmer × *A. glaucum* ($2n = 35$) induced fertility through chromosome doubling. The F_2 plants were exceptionally vigorous, with abundant leafy foliage, and spikes producing an average of 48 seeds each, of which 83% were viable. These amphidiploid plants appear to be perennial, but so far have not been tested for winter hardiness.

The establishment of amphidiploid sectors of sufficient size to make possible the production of seed depended on the successful competition of the amphidiploid with the undoubled tissue. Different hybrids varied greatly in this respect. A relation existed between growth vigour of amphidiploid sectors, or plants, and increase in stomatal size on chromosome doubling.

Eight of eleven F_2 plants possessed 70 chromosomes and the remaining three, 64, 68, and 69. Meiotic studies on five of these plants revealed the formation of 27 to 31 bivalents, 6 to 9 univalents, and occasional trivalents and quadrivalents. Anthers of the amphidiploids dehisced abundant pollen, 94% of which was good, whereas anthers of the undoubled F_1 were unable to dehisce and contained only 2% good pollen.

Introduction

The problem of inducing fertility in sterile intergeneric hybrids in Gramineae by doubling the chromosome number has been receiving considerable attention in recent years. Prior to the discovery of the value of colchicine for this purpose, temperature treatments were found to be moderately successful. The senior author (6) in 1937 doubled the chromosome number in the F_1 of *T. vulgare* var. Kharkov ($2n = 42$) × *A. glaucum* ($2n = 42$) through the application of alternating high and low temperature treatments on the early zygotic division. The resulting 84-chromosome plant lacked vigour and failed to produce spikes prior to publication of the results in December 1938. However, two spikes formed a few months later on clones growing in the greenhouse. These spikes dehisced an abundance of good pollen and a number of plump wheat-like seeds were formed.

The first successful attempt to use colchicine to induce fertility in *Triticum-Agropyron* hybrids was reported in 1939 by Raw (7) who immersed clones of the F_1 of *T. vulgare* × *A. intermedium* in a 0.2% colchicine solution for 10 hr. and obtained one plant that was fully fertile. Sears (8) subsequently reported doubling the chromosome number in sterile hybrids of *T. monococcum* × *Aegilops uniaristata*, *Ae. caudata* × *Ae. umbellulata*, and *Ae. speltoides* × *Ae. umbellulata* by immersing the germinating seeds in 0.05% solution for

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. This contribution forms part of a co-operative investigation on the hybridization of Triticum and Agropyron, undertaken by the Dominion Experimental Farms and the National Research Council of Canada. N.R.C. No. 910.

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24 hr. A high degree of fertility was noted in the amphidiploid sectors of these hybrids.

The present paper gives the results of colchicine treatments on ungerminated F_1 seeds of *Triticum-A. glaucum* hybrids.

Results of Colchicine Treatments

Colchicine treatments were applied in October 1938 to three-year-old seed from crosses of Vernal emmer, Lutescens, and Mindum with *A. glaucum*, and to fresh seed from crosses of Lutescens, Ruby, C.A.N. 1835, and Garnet with *A. glaucum*. The method of seed treatment was similar to that used by Myers (4), who had kindly outlined his procedure prior to publication.

The seed treatments of the various hybrids are given in Table I. The best results were obtained in treatments with 0.2% colchicine for 24 hr. duration. Sixteen seedlings out of 109 appeared to have external symptoms of chromosome doubling. The stomatal sizes of three of these seedlings were determined. At this time Mindum × *A. glaucum* 5-2 appeared to be completely amphidiploid since all the stomata examined in the fourth leaf were abnormally large. About three-quarters of the epidermal leaf tissue of Lutescens × *A. glaucum* 4-1 also appeared to have the doubled complement. In Mindum × *A. glaucum* 5-3, 100 stomata were measured in normal as well as in amphidiploid sectors of the fourth seedling leaf. In the normal sectors, the average length of the guard cells was 63.3 μ, the width 34.6μ, and the

TABLE I

COLCHICINE TREATMENTS OF *Triticum-A. glaucum* HYBRID SEED

Treat. No.	Cross	Colchicine treatment (24 hr.)	No. seeds treated	Total no. seedlings	Seedlings with external symptoms of chr. doubling
1	Vernal × *A. glaucum*	Check	8	8	0
2	Vernal × *A. glaucum*	0.2%	15	6	0
3	Vernal × *A. glaucum*	0.4%	15	2	0
4	Lutescens × *A. glaucum*	0.2%	17	6	1*
5	Mindum × *A. glaucum*	0.2%	14	7	1, 2*, 3*
6	Lutescens 0.62 × *A. glaucum*	0.1%	10	5	7
7	Lutescens 0.62 × *A. glaucum*	0.2%	20	13	12, 13, 14
8	Lutescens 0.62 × *A. glaucum*	0.4%	15	11	25
9	Lutescens 0.329 × *A. glaucum*	0.1%	10	10	36
10	Lutescens 0.329 × *A. glaucum*	0.2%	20	10	46, 47
11	Lutescens 0.329 × *A. glaucum*	0.4%	20	8	0
12	Ruby × *A. glaucum*	0.2%	20	9	1
13	C.A.N. 1835 × *A. glaucum*	0.2%	15	10	1, 2
14	Garnet × *A. glaucum*	0.2%	10	3	0
15	Kharkov × *A. glaucum*	0.2%	28	1	1
	Total		237	119	16

* *Stomatal measurements on these seedlings indicated the presence of sectors possessing doubled chromosome number.*

product 2,176 sq. μ. In the amphidiploid sectors, the average length was 80μ, the width 40.5μ, and the product 3,250μ. The mean product of the length and width in the amphidiploid sectors is 49.4% larger than in the normal sectors. There is no doubt that these clear-cut differences between stomatal dimensions in the same region of the leaf are the result of chromosome doubling.

Epidermal strips were taken from leaves of selected plants at three or four stages during development. It was not possible to strip the epidermis from the entire lower surface of a leaf, particularly in the seedling stages, consequently all the tissue in any leaf could not be examined. This condition improved as the plants grew older and wider strips could be obtained. Measurements of stomata in these strips made it possible to detect tissue with the double chromosome number and to observe its survival during development.

The data obtained from four plants with doubled sectors are given in Table II. In the Mindum × *A. glaucum* hybrids, the doubled (10x) tissue appeared to be able to compete successfully with the normal in the young seedlings, but was rapidly eliminated during later development. In the Lutescens × *A. glaucum* hybrids, the tissue with the doubled (12x) complement appeared to be able to compete more successfully with the undoubled cells throughout development. These data indicate that the survival throughout development of the tissue with the doubled chromosome number differs greatly in the two crosses. Apparently, competition between doubled and undoubled tissue determines the relative amounts of these tissues present in the mature plant.

In addition to stomatal observations it was possible to detect broad strips of doubled tissue by the external appearance of the leaf in certain hybrids, the doubled portions being coarser with crooked veins. In the Lutescens-*A. glaucum* plants at maturity it was possible to see the arrangement of the doubled and undoubled tissue in all the living leaves on the plant. For example, in plant 8-25, half of each blade was completely doubled and the other half was normal, the mid-rib being the dividing line. It was found that the doubled tissue in successive leaves was found alternately on the right and left side of the mid-rib. The fact that the effect was not independent in alternate leaves indicated that the central axis had been similarly affected. Consequently it was expected that half the spike might have the doubled constitution. In spite of these expectations, this particular spike at maturity was completely sterile, indicating that the central axis was not permanently affected.

None of the plants that showed symptoms of chromosome doubling during development produced fertile spikes. However, one Vernal emmer × *A. glaucum* plant (2-12) that failed to show external symptoms of doubling produced nine spikes, one of which was completely fertile, producing 21 seeds. This result emphasizes the fallacy of depending entirely on external symptoms

TABLE II

STOMATAL MEASUREMENTS ON HYBRID PLANTS TAKEN AT DIFFERENT STAGES OF DEVELOPMENT

Cross	Plant No.	Age plants (days)	Stage of development	Epidermis with normal sized stomata: No. of stomatal rows	Epidermis with normal sized stomata: Av. stomatal dimensions, μ, Length	Epidermis with normal sized stomata: Av. stomatal dimensions, μ, Width	Epidermis with abnormally large *stomata*: No. of stomatal rows	Epidermis with abnormally large *stomata*: Av. stomatal dimensions, μ, Length	Epidermis with abnormally large *stomata*: Av. stomatal dimensions, μ, Width	General observations
Mindum × *A. glaucum*	5-2	50	4th leaf	0	—	—	9	82.5	39.9	Leaf apparently completely 10x constitution.
		103	6th leaf	6	63.8	31.9	5	82.5	37.2	Leaf ½ 10x, alternating strips of 5x and 10x tissue.
		103	7th leaf	7	65.2	29.3	4	75.8	37.2	Leaf ½ 10x, alternating strips of 5x and 10x *tissue*.
		147	11th leaf	39	57.2	26.6	0	—	—	Leaf completely of 5x constitution.
Mindum × *A. glaucum*	5-3	50	3rd leaf	3	63.8	31.9	2	85.1	39.9	Leaf app. ½ 10x, alternating strips of 5x and 10x tissue.
		50	4th leaf	6	63.8	34.6	7	79.8	39.9	As 3rd leaf.
		103	7th leaf	22	66.5	30.6	0	—	—	Leaf completely of 5x constitution.
Lutescens × *A. glaucum*	4-1	53	4th leaf	4	61.2	35.9	15	86.4	43.9	Leaf app. ½ 12x, narrow strips of 6x tissue between *wide stripe* of 12x tissue.
		103	7th leaf	4	66.5	27.9	6	83.8	43.9	Leaf app. ½ 12x.
		147	Shot blade	20	58.5	29.3	16	73.1	39.9	Leaf app. ½ 12x. *One side of midrib pure 12x* tissue.
Lutescens × *A. glaucum*	8-25	96	6th leaf	9	67.8	35.9	3	91.8	43.9	Leaf app. ½ 12x, narrow strips of 12x dispersed between 6x tissue.
		140	Shot blade	34	54.5	29.3	3	75.8	39.9	Leaf app. ½ 12x, narrow strips of 12x dispersed between *6x tissue*.

for the detection of amphidiploid tissue, since it may be entirely normal in appearance, and increase in cell size would only be apparent on microscopic examination.

Development and Fertility of Amphidiploid Vernal emmer × *A. glaucum*

The seed produced on the fertile F_1 spike was compared with the two parents. The weight per 1000 kernels in grams for Vernal emmer is 34.2, for the F_1 16.6, and for *A. glaucum* is 5.7 (Fig. 2). The amphidiploid hybrid seed is thus about three times as heavy as the seed of the grass parent and one-half that of the wheat parent. Twelve seeds were sown in the spring of 1939 and all germinated. Chromosome numbers were determined for 11 of the plants, either by root tip or p.m.c. examination. Eight plants had 70 chromosomes, a number, of course, exactly double that of the normal F_1, and the three other plants had 64, 68, and 69 chromosomes.

Twelve F_2 seeds were sown on May 16, 1939, and transplanted to the garden early in June, where they grew vigorously.* They produced an abundance of leafy foliage of slightly coarser texture than that of the undoubled F_1, but as fine or finer than that of most *Triticum-A. glaucum* hybrids. By late autumn flowering spikes were developing freely, and a total of 503 seeds had developed by mid-October when it was necessary to harvest the material. Detailed data on the fertility and germinability for nine of the 11 spikes harvested are given in Table III. In spite of the lateness of maturity a remarkably good seed set was obtained. An average of 48 seeds per spike was produced, which is believed to be in excess of *A. glaucum* and would compare favourably with Vernal emmer. Counts were made on the number of well developed florets per spike and the percentage fertility was based on this. An average of 77% of the florets produced seed, two of the plants (2-12-1 and 2-12-7) being definitely superior in this regard. The fourth

TABLE III

FERTILITY OF THE F_2 AND VIABILITY OF F_3 SEED OF AMPHIDIPLOID PLANTS OF VERNAL EMMER × *A. glaucum*

Plant No.	Number spikes examined	Number spikelets	Number florets	Number seeds	Fertile florets, %	Germination, %
2-12-1	5	104	321	260	81.0	87.2
2-12-5	1	19	45	27	60.0	88.9
2-12-7	2	42	142	115	81.0	75.2
2-12-9	1	22	61	34	55.7	88.2
Total	9	187	569	436		
Average		21 per spike	63.2 per spike	48.4 per spike	76.6	83.5

* *In the autumn of 1939, 30 F_2 and 254 F_3 seeds of the Vernal-A. glaucum amphidiploid were supplied to the Division of Forage Plants, Central Experimental Farm, Ottawa. The resulting plants showed considerable promise, as reported in Magazine Digest, May, 1940. An additional 6,400 seeds were supplied in April, 1940.*

floret was fertile in 43% of the spikelets of 2-12-7 and in 18% of the spikelets of 2-12-1, whereas neither of the remaining two plants (2-12-5 and 2-12-9) had any fertile fourth florets. However, undue emphasis should not be placed on the relative seed production in the year of seeding. In the second year the seed should ripen much earlier and under these more favourable conditions it is expected that fertility, plumpness of seed, and total yield of all the plants will be greatly increased.

The germination data in Table III show that the viability of the seed is satisfactory, especially since some of it was immature when harvested.

The colchicine-treated F_1 plant (2-12) that produced the fertile spike was transplanted to the garden in the spring of 1939 and it again produced one fertile spike (21 seeds) and 20 sterile spikes. The plant was cloned in the autumn into 16 portions in an endeavour to isolate the amphidiploid sector.

A comparison of stomatal size in doubled and undoubled leaf tissue of Vernal × *A. glaucum* is shown in Table IV and Figs. 3 and 4. There is a striking increase in the length and width of the guard cells and the area index of the 70-chromosome plant is 109% greater than that of the 35-chromosome plant.

TABLE IV

COMPARISON OF STOMATAL SIZE OF DOUBLED AND UNDOUBLED LEAF TISSUE

Hybrid	Plant No.	Chr. no. ($2n$)	No. stomata examined	Mean length (μ)	Mean width (μ)	Area index ($L \times W$)
Mindum × *A. glaucum* (undoubled sector)	3	35	100	63.3	34.6	2,176
Mindum × *A. glaucum* (doubled sector)	3	70	100	80.0	40.5	3,250
Vernal × *A. glaucum* (undoubled F_1)	2-12	35	100	53.1	27.8	1,476
Vernal × *A. glaucum* (doubled F_2)	2-12-8	70	100	86.0	35.8	3,083

The data in Table V indicate a relation between growth vigour of amphidiploid sectors or plants, and increase in stomatal size on chromosome doubling. From this it is concluded that the more vigorous the amphidiploid tissue the greater is the increase in stomatal size on chromosome doubling. This relation is most obvious in the comparison of the Kharkov × *A. glaucum* with the Vernal × *A. glaucum*, but is also indicated by the relative ability of the doubled tissue to compete successfully with the undoubled tissue in the other two crosses.

The amphidiploid Vernal × *A. glaucum* material appears to have excellent agronomic possibilities, although little should be said on this point until extensive field trials have been completed. However, it has one possible fault in that the rachis shatters on threshing like the Vernal parent. The seeds are not held tightly in the glumes and close threshing may free them.

TABLE V

RELATION OF VIGOUR OF AMPHIDIPLOID SECTORS OR PLANTS TO INCREASE IN STOMATAL SIZE ON CHROMOSOME DOUBLING

Hybrids	Increase in area index on chr. doubling, %	Observations on vigour
Kharkov × *A. glaucum*	36	Amphidiploid plants less vigorous than sterile F_1.
Mindum × *A. glaucum*	49	Doubled tissue (10x) unable to compete successfully with undoubled tissue.
Lutescens × *A. glaucum*	78	Doubled tissue (12x) able to compete successfully with undoubled tissue.
Vernal × *A. glaucum*	109	Amphidiploid plants more vigorous than sterile F_1.

If this is not possible, it will be necessary to sow the whole spikelet, which will be wasteful as it may contain up to four seeds. It should be possible to breed away from this spike character by crossing with other 70-chromosome amphidiploids. In an effort to produce other amphidiploids for this purpose, seed and seedlings of 12 additional *Triticum-A. glaucum* hybrids have recently been treated with colchicine.

The excellent leafiness, tillering capacity, and vigour of the amphidiploid are illustrated in a photograph (Fig. 1) of plant 2-12-1 taken on November 2, 1939. The height is indicated by the yard stick. The plants show definite indication of being perennial as their clones are growing vigorously. Winter hardiness has not yet been tested, but the sterile F_1 is known to be reasonably winter hardy in Ottawa and the more vigorous amphidiploid derivative is not likely to be different in this respect.

Cytological and Pollen Observations

It was previously reported (5) that the sterile F_1 of Vernal emmer × *A. glaucum* possessed an average of 20.4 univalents, 6.2 bivalents, and 0.8 trivalents per nucleus. The low degree of homology between the *Triticum* and *Agropyron* chromosomes undoubtedly accounts for the complete sterility of the undoubled F_1 generation. Four meiotic stages in this material are illustrated in Figs. 5–8. The univalents lag at first anaphase and divide equationally. In the second division the half univalents wander at random and those that do not happen to be included in the tetrad nuclei form micronuclei which subsequently degenerate.

On doubling the chromosome number in the F_1 of Vernal emmer × *A. glaucum*, each chromosome should have a completely homologous partner and 35 bivalents would be expected. These expectations were not realized, since in the five F_2 plants examined (Table VI) averages of from 27 to 30 bivalents were found. The failure of all the chromosomes to pair cannot be attributed to lack of homology but rather to the lack of opportunity for

TABLE VI

SUMMARY OF MEIOTIC CHROMOSOMAL ASSOCIATIONS FOR F_2 VERNAL EMMER × *A. glaucum* AMPHIDIPLOIDS

Plant No.	No. of cells	Associations of				P.M.C. count
		I	II	III	IV	
2-12-1	20	7.3	30.7	0.1		69
2-12-2	20	8.7	27.55		0.05	64
2-12-5	20	8.0	30.6	0.2	0.05	70
2-12-7	20	6.0	30.9		0.05	68
2-12-8	20	8.65	30.25	0.15	0.10	70

all of the homologous chromosomes to pair during the zygotene stage, at which time homology presumably results in pairing. It appears reasonable to suppose, as also suggested by Kostoff (2), that the difficulty of pairing would be increased on doubling of the chromosome number. It follows that there must be some limit in chromosome number beyond which zygotene pairing becomes difficult in spite of perfect homology. In the Vernal × *A. glaucum* amphidiploid nucleus possessing 70 large chromosomes, either the duration of the zygotene stage or the pairing attraction may be inadequate to overcome the difficulties caused by the increase in spatial distribution of the homologous chromosomes.

Investigations of Berg and Oehler (1) and Müntzing (3) indicate that genetic factors influence pairing conditions in *Triticale*. Müntzing suggests that the main cause of the meiotic lability in *Triticale* is an automatic inbreeding degeneration of the rye component. His explanation would not apply to the situation in the Vernal × *A. glaucum* amphidiploid in which both parents are largely self-fertilized and the material studied has been inbred for only one generation. However, genetic factors capable of causing variation in pairing conditions possibly exist in this material, and forms in which a high percentage of the chromosomes form bivalents may eventually be obtained by selection.

It has not been possible to obtain p.m.c. material from the amphidiploid sector of the F_1 plant, but it can be assumed that similar meiotic irregularities existed as indicated by the variable chromosome numbers in the F_2 noted above.

In spite of the differences in the chromosome number of the F_2 plants examined cytologically, there were only small differences in the frequency of the various configurations both within and between plants. For example, two plants had 26-33 bivalents and others had 27-33, 28-33, and 25-30 bivalents per nucleus. The p.m.c. material of plant 2-12-2, which had the lowest mean number of bivalents (27.5) and the lowest range (25-30), was collected on October 13, at least two weeks later than any other material examined. Thus it is probable that the cool weather conditions existing at the time this material was collected may have influenced pairing.

The number of multivalent configurations was much lower than the theoretical expectation on the basis of pairing in the undoubled F_1. Since an average of six bivalents per nucleus was found in the sterile F_1, it should be possible for six quadrivalents per nucleus to appear in the doubled F_2. Actually only 9 trivalents and 5 quadrivalents were observed in the hundred nuclei examined, which indicates that the chromosomes pairing in the undoubled F_1 were only partially homologous. It is also of interest to note that 10 out of the 14 multivalent configurations occurred in the two 70-chromosome plants.

Meiosis in the fertile amphidiploid is shown in Figs. 9–16. The range in bivalent formation is illustrated by Figs. 9 and 10. In the former there are 10 univalents and 27 bivalents, while in the latter there are two univalents and 33 bivalents. The behaviour of the univalents is similar to that found in the undoubled F_1. The lagging and splitting of univalents in the first division is illustrated in Figs. 11–13. The movement at random of the half univalents in the second division is shown in Fig. 14 and the resulting micronuclei in the tetrads are shown in Fig. 15. The average number of micronuclei per tetrad in four plants is recorded in Table VII. The four plants were very similar in this respect and averaged approximately four micronuclei per tetrad. In spite of the relatively frequent occurrence of micronuclei, the pollen appeared to be very good. (Fig. 16).

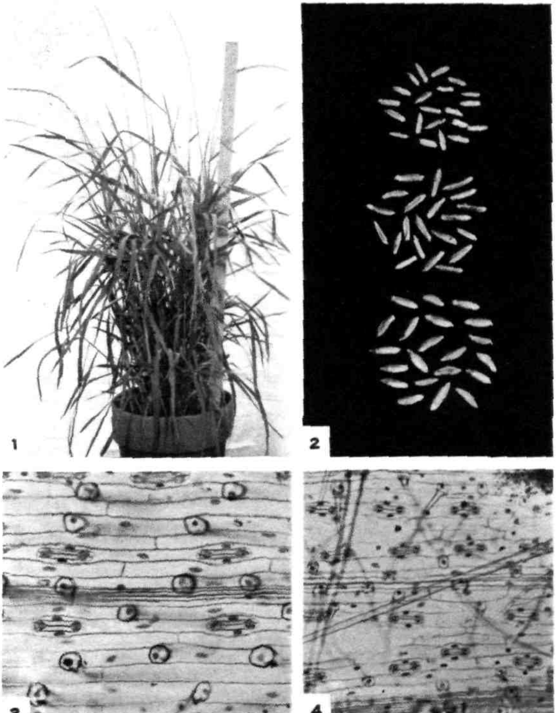
TABLE VII

MICRONUCLEI IN TETRADS OF F_2 AMPHIDIPLOID VERNAL EMMER × *A. glaucum* PLANTS

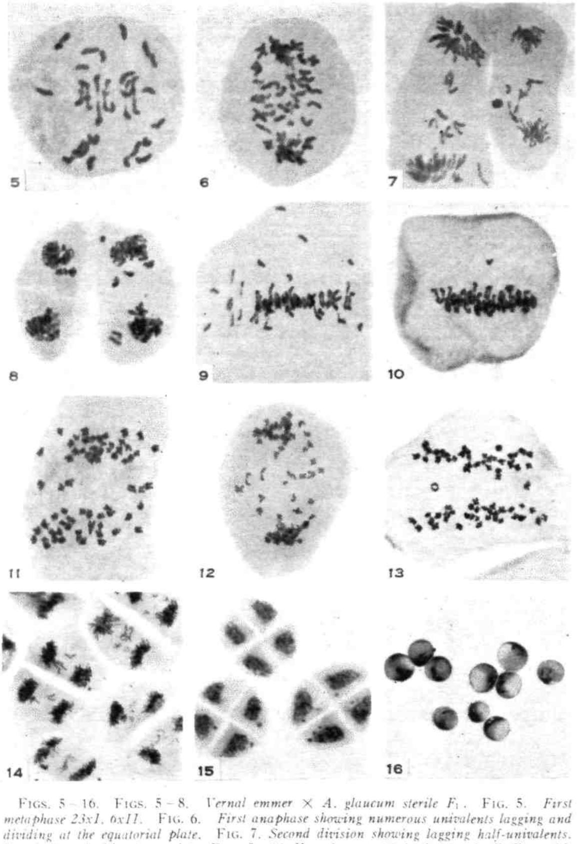
Plant No.	No. tetrads	No. micronuclei	Average no. per tetrad
2-12-1	40	144	3.6
2-12-5	40	149	3.7
2-12-7	40	186	4.6
2-12-8	40	173	4.3
Total	160	652	
Average			4.08

A detailed study of the pollen of five F_2 plants and the undoubled sector of the F_1 plant was made. The pollen was graded on the basis of the number of nuclei present and as to whether the quantity of the cytoplasm was normal, reduced, slight or none. The individual amphidiploid plants were very similar in that a high proportion (92 to 97%) of their pollen grains had three nuclei and the normal amount of cytoplasm. Consequently only the summary of all the plants is shown in Table VIII. A total of 472 out of 500 amphidiploid pollen grains (94%) possessed three nuclei and the normal amount of cytoplasm, whereas less than 2% of the pollen grains of the undoubled F_1 could be considered normal. The proportion of good pollen

PLATE I



FIGS. 1 – 4. FIG. 1. *F_2 amphidiploid Vernal emmer × A. glaucum 2-12-1.* FIG. 2. *Seeds: above, A. glaucum; below, Vernal emmer; centre, amphidiploid F_1 Vernal emmer × A. glaucum.* FIG. 3. *Stomata of F_2 amphidiploid Vernal emmer × A. glaucum 2-12-8.* FIG. 4. *Stomata of the sterile F_1 Vernal emmer × A. glaucum. Magnification of Figs. 3 and 4, ×135.*



FIGS. 5 – 16. FIGS. 5 – 8. *Vernal emmer* × *A. glaucum sterile* F_1. FIG. 5. *First metaphase 23xI, 6xII.* FIG. 6. *First anaphase showing numerous univalents lagging and dividing at the equatorial plate.* FIG. 7. *Second division showing lagging half-univalents.* FIG. 8. *Tetrad with micronuclei.* FIGS. 9 – 16. *Vernal emmer* × *A. glaucum fertile* F_2 *amphidiploids.* FIG. 9. *First metaphase of 2-12-2, 10xI and 27xII.* FIG. 10. *First metaphase of 2-12-7 with 2xI and 33xII.* FIG. 11. *First anaphase of 2-12-8* ($2n = 70$). FIG. 12. *Late first anaphase of 2-12-2, dividing univalents.* FIG. 13. *First anaphase 2-12-7* ($2n = 68$). FIG. 14. *Lagging half-univalents in second anaphases 2-12-8.* FIG. 15. *Tetrads with micronuclei 2-12-7.* FIG. 16. *Pollen 2-12-1. Aceto-carmine smear preparations. Magnification of Figs. 5 – 8 is ×680, of Figs. 9 – 15 is ×410, of Fig. 16 is ×85.*

TABLE VIII

SUMMARY OF POLLEN OBSERVATIONS ON AMPHIDIPLOID F_2 AND UNDOUBLED F_1 OF VERNAL × *A. glaucum*

Plant No.	Cytoplasm	Number of nuclei				Total	Per cent
		3	2	1	0		
2-12-1, 2-12-5, 2-12-7, 2-12-9, 2-12-10	Normal	472	2	0	0	474	95.8
	Reduced	8	1	0	1	10	2.0
	Slight	1	3	0	2	6	1.2
	None	0	0	0	10	10	2.0
	Total	481	6	0	13	500	
	Per cent	96.2	1.2	0.0	2.6		100
Undoubled F_1 (2-12)	Normal	4	9	4	0	17	6.8
	Reduced	0	2	31	0	33	13.2
	Slight	2	3	75	19	99	39.6
	None	0	0	0	101	101	40.4
	Total	6	14	110	120	250	
	Per cent	2.4	5.6	44.0	48		100

in the undoubled F_1 was so low that dehiscence never occurred, while the F_2 plants dehisced an abundance of good pollen.

Acknowledgments

The authors are greatly indebted to Dr. T. M. Stevenson, Dr. J. M. Armstrong, and Mr. A. McLennan of the Division of Forage Plants, Central Experimental Farm, Ottawa, for supplying hybrid seed, and for generous co-operation at all times.

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STUDIES ON BROWNING ROOT ROT OF CEREALS

VI. FURTHER CONTRIBUTIONS ON THE EFFECTS OF VARIOUS SOIL AMENDMENTS ON THE INCIDENCE OF THE DISEASE IN WHEAT[1]

By T. C. Vanterpool[2]

Abstract

Further work has substantiated earlier findings that phosphatic fertilizers and farm manure will give adequate control of *Pythium* root rot of wheat in infested prairie soils. The improvement in growth resulting from these amendments is considered to be due to the production of a larger number of quicker growing roots which lessens the chances for infection and leaves more roots healthy, though the same percentage may be affected as in diseased plants showing severe leaf discolorations. Experiments have failed to indicate that the phosphatic materials increase resistance appreciably. Nitrogenous materials when applied singly had virtually no effect on growth, but once ample phosphorus was added, further nitrogen applications gave substantially greater increases than phosphate alone. Phosphorus is apparently the chief limiting element. No difference was found in preliminary tests in the phosphate-fixing power of browning and normal soils. Typical browning soils responded irregularly to small applications of boron, copper, manganese, or zinc, but were not found to be seriously lacking in these elements. Moderate benefits resulted from heavy applications of gypsum and of sulphur. Browning soil was found also to be deficient in phosphate for non-cereals such as alfalfa, buckwheat, carrots, flax, lettuce, and sweet clover. These crops were not attacked by the *Pythium* spp. pathogenic to cereals. *Consequently the poor growth of the non-cereals in* browning soil appears to be due to nutrient deficiencies, while the poor growth of cereals is due to both root-destroying fungi and nutrient deficiencies. In both instances phosphorus is probably the chief limiting element. Ground cereal straw, sweet clover hay, and weed hay amendments gave moderate increases in the growth of wheat. No consistent differences were found in the carbon-nitrogen ratios of browning and normal soils. The results as a whole suggest that two of the most practicable means of meeting the browning root-rot situation are, firstly, to supply supplemental nutrients in the form of artificial fertilizers, and secondly, to add organic residues or farm manure regularly to fields subject to the disease.

Introduction

It has been found difficult to reduce and impossible to destroy under field conditions the species of *Pythium* commonly found attacking wheat roots on the Canadian prairies. Thus both *Pythium arrhenomanes* Drechsl. and *P. tardicrescens* Vanterpool, the two species most frequently isolated from browning root-rot lesions in recent years, have repeatedly been obtained from the roots of wheat seedlings grown in soils that had been in clean fallow for ten years or more. It is not known whether the resting oospores of these species of *Pythium* are capable of retaining their vitality for that length of time in the soil, or whether these fungi can live saprophytically as members of the natural soil microflora. Their poor competitive abilities with other

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Contribution from the Laboratory of Plant Pathology, University of Saskatchewan, Saskatoon, with financial assistance from the Saskatchewan Agricultural Research Foundation.
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micro-organisms in artificial culture does not lend support to the latter view. According to Carpenter (5) the species of *Pythium* causing root rot of sugar-cane, which is also pathogenic on wheat, may grow saprophytically in the soil, but it is not a vigorous competitor with the strictly saprophytic soil fungi. Recontamination of fallowed land by wind-blown oospores during dust storms probably occurs quite frequently, as is indicated by the finding of the pathogens in the roots of wheat seedlings grown in soil collected from deep drifts some distance from cultivated fields, and in soil from the eaves-troughs of city houses in Saskatoon.

As it has not been feasible to starve or destroy the root-infecting fungi by any practicable farm operations, studies on the improvement of soil conditions and other factors which might (i) lead to the development of more vigorous plants, and (ii) tend to check the activity phase of the parasites, were accordingly undertaken. Some results on the effects of various soil amendments on the root rot have been reported in Part IV (20) of this series. The earlier experiments have been repeated and extended and the results used as a basis for this paper.

Methods Used in the Greenhouse Investigations

The most feasible method of studying browning root rot experimentally is to use naturally-infested field soil and conduct the experiments under controlled conditions in the greenhouse, or out-of-doors.

The soil is collected in June from severely attacked fields; the composite of *Pythium*-infested soil from diseased areas forms the stock of browning soil, and the composite from healthy areas forms the stock of normal soil. Quantities of these soils are taken as required and filled into six-inch containers. Artificial fertilizer applications are incorporated into the soil at a distance of 1 in. below to 1 in. above seed level, while the organic amendments are mixed through the top 4 in. of soil. Marquis seed of good quality is sown at a depth of 2 to $2\frac{1}{2}$ in. to permit the development of crown roots at some distance from the seminal roots so as to facilitate counting. Twelve seeds are sown to each six-inch pot or one-gallon crock and later thinned out to ten; this eliminates any overcrowding effects up to the five- to six-weeks stage when browning symptoms are most conspicuous. The pots are randomized and rerandomized from time to time during the course of the experiment. Supplemental overhead illumination is necessary during the winter months. About once a week watering is done from below by placing the containers in water to a depth of 1 in. until the surface soil becomes moist; at all other times watering is done from above. In studies on comparative methods of watering, the one just outlined has been found most suitable for experiments on root rots. Oven-dry weights refer to the weights of the tops. Root-lesion counts are confined to the crown roots because of the impracticability of estimating visually the amount of disease on the fine seminal root system.

Commercial Fertilizer Amendments and Wheat Response

In experiments reported previously, Vanterpool (20, Part IV) showed that phosphatic amendments permitted the development of normal wheat plants in soil naturally infested with *Pythium*, while inorganic nitrogenous fertilizers usually had no effect. In the following experiments, materials used previously have been tested further and new ones included.

1. Various Fertilizers and Fertilizer Combinations

Experiment 1. The phosphatic fertilizers were applied at rates equivalent to 100 lb. of phosphorus pentoxide per acre, the nitrogenous fertilizers at 96 lb. of nitrogen per acre, and the potassic fertilizers at 98 lb. of potassium oxide per acre. The materials were mixed into the soil at seed level, as previously described, at the time of planting. The seedlings were harvested after six weeks, by which time browning symptoms were well advanced in the controls.

In Experiment 1, Table I, phosphorus applied singly as triple superphosphate has given only a moderate increase, though much larger increases are usual, as will be seen from the results in Experiment 2, Table I, and from

TABLE I

THE EFFECT OF FERTILIZERS ON THE GROWTH OF WHEAT SEEDLINGS IN NATURALLY INFESTED FIELD SOIL

Treatment	Height, cm.	Seminal roots per plant	Crown roots			Dried plants	
			Total no.	No. healthy	Diseased, %	Weight, gm.	Increase, %
*Experiment 1**							
None—control	44	—	21	13	38.1	6.7	—
Triple superphosphate (1-43)	48	—	59	35	40.6	9.2	37.3
Ammonium phosphate (11-48)	52	—	73	44	39.7	13.5	101.5
Ammonium phosphate (16-20)	*56*	—	*80*	*40*	*50.0*	*15.4*	*129.8*
Ammonium sulphate	48	—	21	9	42.8	8.1	20.9
Ammonium nitrate	45	—	17	7	58.8	8.0	19.4
Calcium nitrate	44	—	22	10	54.5	7.0	4.5
Sodium nitrate	45	—	15	6	60.0	7.1	5.9
Potassium nitrate	47	—	23	18	21.7	7.7	14.9
Urea	47	—	23	16	30.4	8.1	20.9
Cyanamide	46	—	28	16	42.8	7.1	5.9
Potassium sulphate	44	—	21	13	38.1	6.9	2.9
Phosphorus + potassium	51	—	60	30	50.0	10.9	62.7
Nitrogen + phosphorus + potassium	53	—	88	49	44.3	14.2	111.9
Experiment 2†							
None—control	36	5.00	132	28	78.8	5.47	—
Triple superphosphate (1-43)	47	5.16	262	77	70.6	13.50	146.8
Ammonium phosphate (11-48)	48	5.24	298	74	75.2	15.99	192.3
Ammonium phosphate (16-20)	48	5.06	335	88	73.7	21.05	284.8
Ammonium sulphate	42	4.84	140	43	69.3	6.80	24.3

* *October 27 to December 8, 1938. Four replicates to each treatment.*
† *February 11 to March 25, 1938. Five replicates to each treatment.*

data previously published (20). Nitrogen applied singly as inorganic or organic nitrogenous fertilizer has given a slight response, and potassium no response. When phosphorus and nitrogen are applied in combination, the increase is much greater than with phosphorus alone. The nitrogen-phosphorus combination gave greatest increase, phosphorus-potassium next, and nitrogen-potassium a slight increase, indicating that phosphorus is in greatest deficiency. Once the deficiency of phosphorus is corrected, additional nitrogen gives increased yields.

It will be observed that of the materials supplying nitrogen alone, the acid nitrogenous fertilizers, ammonium nitrate, ammonium sulphate, and urea, gave slightly better response than the basic fertilizers, calcium nitrate, sodium nitrate, cyanamide, and potassium nitrate.

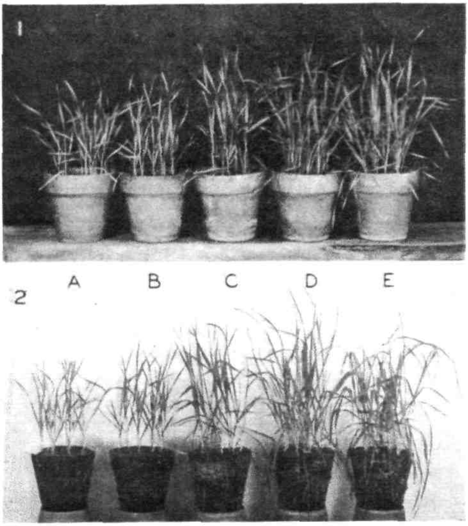
The complete fertilizer gave approximately the same increase as the ammonium phosphates, again indicating that ample potassium is present in infested soil.

No definite correlation is evident between the percentage of diseased crown roots and the dry weights of the plants. The impracticability of estimating accurately the amount of disease on the fine laterals of both seminal and crown roots adds to the difficulty of arriving at a disease rating that correlates closely with the amount of growth. Dry weight appears to be related to the number of healthy crown roots. Thus the 16-20 ammonium phosphate, with over three times as many healthy crown roots as the control series but with a larger percentage diseased, has produced more than twice as much plant growth.

Experiment 2. This was conducted in essentially the same manner as Experiment 1, except that the soil used had its viable *Pythium* content raised by previously growing in it a crop of wheat seedlings for five weeks, and then removing the seedlings by shaking the soil off the roots. If the next crop is sown at once before allowing the soil to dry out, an increased amount of viable inoculum is present in the fine rootlets broken off in the harvesting operations just described.

The results of Experiment 2, Table I, are similar to those in Experiment 1 except that the percentage increases from the three phosphates are greater (Figs. 1 and 2). Several factors are probably responsible for this, such as the increase in inoculum intensity, a decrease in nitrate resulting from the previous crop, and a better utilization of the phosphates because of the improved light conditions (cf. 3). There is a tendency for the phosphate to increase the number of seminal roots slightly, but not significantly.

The phosphates, especially those containing nitrogen, usually prevented, but occasionally only delayed, the appearance of leaf yellowing at the six-weeks stage when the plants were harvested. They increased the number and length of crown roots (20, p. 238), the number of tillers (Fig. 1), and the dry weights of the plants; and, in fact, gave practical control of the disease.



FIGS. 1 AND 2. *The effect of nitrogen and phosphorus on the growth of wheat in soil naturally infested with Pythium.* A, *untreated;* B, *ammonium sulphate;* C, *triple superphosphate;* D, *ammonium phosphate (11-48); and* E, *ammonium phosphate (16-20). The beneficial effect of phosphorus on the development of fine lateral roots is well brought out in Fig. 2.*

2. PHOSPHATE CARRIERS

An experiment was conducted to ascertain the efficiency of various phos phate carriers as determined by the percentage increase in dry weight o fertilized plants over untreated controls in infested soil (Table II). Ther were four pots to each treatment. Chemically pure phosphates were usec and applied at the rate of 100 lb. per acre.

The ammonium dihydrogen phosphate eliminated above-ground brownin symptoms. The highly beneficial effects of this compound are due to th additional nitrogen it contains, as it has already been shown (Table I) tha ammonium phosphates give the best response. Calcium as a carrier woul be expected to be better than potassium, as calcium is known to inhibit th disease (Section 4 below), while potassium makes no appreciable differenc (Experiment 1). Sodium appears to be a poor carrier of phosphorus fo browning soils.

TABLE II

THE RELATIVE EFFECTS OF VARIOUS PHOSPHATE CARRIERS ON THE GROWTH OF WHEAT SEEDLINGS IN BROWNING SOIL

February–March, 1939

Treatment	Increase in dry weight over controls, %
Ammonium dihydrogen phosphate	125.4
Calcium dihydrogen phosphate	49.1
Potassium dihydrogen phosphate	39.0
Sodium dihydrogen phosphate	25.4

3. Phosphate Fixation

Cooke (6) has indicated that the *Pythium*-infested sugarcane soils of Hawaii are high fixers of added phosphate. Fourteen sample pairs of *Pythium*-infested wheat soils and normal soils of Saskatchewan were tested for their phosphate-fixing power. The results were irregular, neither soil series showing a greater tendency for phosphate fixation. In unpublished work, A. W. Hoffer, student assistant, has shown that whereas available phosphorus is usually lower in the infested soil (cf. 20, Part III), the total phosphorus in the two soils shows no such relation. The averages for total phosphorus were approximately the same in each series. The problem as to why diseased areas having the same total phosphorus should have more of it in an unavailable condition is one that should be given attention by the soil microbiologists and soil chemists.

4. Calcium Sulphate

Preliminary experiments had shown clearly the beneficial effects of finely ground gypsum ($CaSO_4 . 2H_2O$) in inhibiting the root rot. In Table III, data are given on the effects of gypsum and triple superphosphate singly and

TABLE III

THE EFFECT OF GYPSUM AND TRIPLE SUPERPHOSPHATE ON THE GROWTH OF WHEAT IN BROWNING SOIL

November 18 to December 30, 1938

Treatment	Dried plants	
	Weight, gm.	Increase, %
Browning soil—untreated	2.50	—
Gypsum, 0.7%	3.92	56.8
Gypsum, 1.4%	4.80	92.0
Gypsum, 2.8%	3.92	56.8
Triple superphosphate, 100 lb. per acre	4.60	84.0
Gypsum, 1.4% + triple superphosphate	7.35	194.0

TABLE IV

CHEMICAL ANALYSES OF PAIRED SAMPLES OF SOIL FROM BROWNING AND NORMAL AREAS OF WHEAT ON SUMMERFALLOWED LAND*

1937 and 1938

Sample location	Available P, p.p.m.		NO_3, p.p.m.		Exchangeable bases						Total bases, m.e.		Ca/Mg	
					Ca, m.e.		Mg, m.e.		Na, m.e.					
	Browning	Normal	Browning	Normal	Browning	Normal	Browning	Normal	Browning	Normal	Browning	Normal	Browning	Normal
1937:														
Prudhomme	52	79	38.2	30.3	16.4	28.6	13.5	14.9	—	—	—	—	1.21	1.92
Cudworth	42	67	27.8	50.5	21.2	27.6	9.5	13.1	—	—	—	—	2.23	2.10
Leofnard	56	114	18.3	9.0	15.9	16.5	7.6	4.7	—	—	—	—	2.09	3.51
Wakaw	59	77	16.2	24.7	11.1	27.0	3.3	7.9	—	—	—	—	4.27	3.42
?	54	93	29.4	13.0	24.8	37.0	9.8	9.3	—	—	—	—	2.52	3.98
St. Gregor	56	84	15.4	14.4	25.9	27.7	9.8	14.3	—	—	—	—	2.64	1.93
Composite	—	—	—	—	23.1	38.3	8.9	7.1	2.4	1.9	34.4	47.3	2.59	5.30
Plunkett	—	—	—	—	19.0	25.3	25.5	6.0	1.8	1.9	46.3	33.2	0.74	4.22
Cudworth	—	—	—	—	32.4	41.6	10.1	14.1	1.9	1.8	44.4	57.5	3.21	2.95
Wakaw	—	—	—	—	36.1	68.9	18.6	8.9	3.1	4.3	57.8	82.1	1.94	7.74
1938:														
Lanigan	66	66	5.9	1.4	26.2	17.7	7.1	7.5	1.3	1.3	34.6	26.5	3.69	2.36
Fenton	27	140	28.0	33.2	27.9	37.4	14.6	20.6	1.3	2.0	43.8	60.0	1.91	1.81
Saskatoon	60	82	5.2	3.6	15.7	17.3	11.0	11.1	1.9	1.5	28.6	29.9	1.43	1.56
Composite	46	67	41.9	18.8	22.1	29.6	8.8	12.3	1.6	1.7	32.5	43.6	2.51	2.40
Humboldt	28	35	25.7	20.2	12.2	20.6	—	—	—	—	—	—	—	—
Lanigan	50	60	2.3	1.7	7.7	17.7	—	—	—	—	—	—	—	—
Lanigan	13	22	2.6	2.4	9.6	9.6	—	—	—	—	—	—	—	—
Saskatoon	22	130	19.2	8.3	9.3	9.7	—	—	—	—	—	—	—	—
Saskatoon	59	72	11.2	14.2	8.9	21.3	—	—	—	—	—	—	—	—
Hagen	38	54	20.6	13.6	15.0	16.2	—	—	—	—	—	—	—	—
Selkirk, Man.	65	91	1.4	1.0	21.5	22.4	—	—	—	—	—	—	—	—
Mean	46.6	78.4	18.2	15.3	19.3	26.6	11.3	10.8	1.9	2.0	40.3	47.5	2.36	3.23

* *Thanks are due to G. Dion, formerly of the Soils Department, for making these determinations.*

in combination on the growth of wheat in a composite soil sample of browning soil. The gypsum was mixed into the whole mass of soil in the pots at rates of 0.7, 1.4, and 2.8% of the weight of the soil. Each treatment was in triplicate. The analysis of the untreated soil is given under browning soil in the 1938 Composite sample in Table IV.

Both the gypsum and triple superphosphate amendments gave substantial increases in the growth of wheat in browning soil. The combined amendments produced a significantly greater increase in dry weight than either component alone. Similar results were obtained by Albright and Klemme (1) from phosphates and limestone on the growth of Korean Lespedeza. These workers were of the opinion that the limestone mobilized the phosphates into the Lespedeza. Carpenter (5) has reported that calcium, as well as phosphorus, is beneficial to sugarcane in *Pythium*-infested soils in Hawaii.

The response from calcium is interesting and difficult to explain since the exchangeable calcium in browning soils, as determined by the ammonium acetate extract method, seems to be sufficient for the plants' needs (Table IV). The improvement brought about by the gypsum may possibly be due to one or more of the following: its increase in phosphate solubility, its effect on the physical condition of the soil, and its sulphur content; for, as reported later, sulphur applications are also beneficial. It is possible that the improvement in these soils derived from the commercial phosphatic amendments may be due partly to the sulphate that they contain. The results in Table II, however, show that high increases can be obtained from ammonium dihydrogen phosphate, which contains no sulphur.

It will be seen from Table IV that the average results of the analyses of 21 pairs of samples show the browning soils to contain 19.3 milliequivalents of exchangeable calcium and the normal soils 26.6 m.e. of calcium. In only one pair out of 21 is the exchangeable calcium higher in the browning soil. This is probably correlated with the usually lower available phosphorus and slightly lower pH of the infested soils (cf. 20). The results for available phosphorus and nitrate are in agreement with those reported earlier (20), and are included here for comparison with the data on exchangeable bases. No consistent differences in exchangeable magnesium, exchangeable sodium, or the calcium-magnesium ratio can be detected in the paired samples. Of the normal soils, 75% are higher in total exchangeable bases, but not appreciably.

5. PHOSPHATE IN RELATION TO *Pythium* INFECTION

It has been found extremely difficult to inoculate sterile soil uniformly with *Pythium* so that pot experiments could be conducted on a large scale, as is possible to a reasonable degree with *Fusarium* and *Helminthosporium* spp. This practically eliminates suitably replicated pot experiments to determine the specific effect of phosphate on *Pythium* infection of wheat roots. Wheat seedlings were accordingly grown in root-study boxes in sand cultures low in phosphate and high in nitrate, high in phosphate and low in nitrate, no

phosphate, and in full nutrient solutions, and the roots inoculated with agar discs of *Pythium* inoculum.

Under the conditions of the experiments the fungus attacked the roots at approximately the same rate regardless of treatment; that is, no significant differences could be recognized. It seems that the phosphate has little, if any, effect on increasing the resistance of the tissue, but that the phosphate, by *stimulating root development* both as regards numbers and rate of growth, helps the plants to escape infection. Thus it is found that phosphate-treated plants may have the same percentage of diseased crown roots as untreated plants in browning soil, but probably because of the larger total number and greater length of healthy crown roots, the phosphate-treated plants have been able to develop normally in spite of the diseased roots.

Greaney (9) found that deficiencies in phosphate did not affect the disease incidence of wheat plants to root rot caused by *Fusarium culmorum* (W. G. Sm.) Sacc. His results suggest that the effect of phosphatic fertilizers is more important on plant growth than on the severity of infection by *F. culmorum*. Sideris and Paxton (18) are of the opinion that resistance of pineapples to *Pythium arrhenomanes* is determined by the ability of infested plants to produce new roots and thus perpetuate the life of the plant, and susceptibility by the converse of this condition. Carpenter (5) regards susceptibility of sugarcane to *Pythium* root rot as due either to a natural weakness of the variety to attack or to a preconditioning of the roots of naturally resistant varieties by malnutrition. Certain varieties become more susceptible where the soil nitrogen is in excess, while other varieties are more readily attacked where phosphate and calcium are deficient.

6. Trace Elements

With the occasional exception of boron and zinc, trace elements have made no appreciable difference in the growth of wheat when applied to browning root-rot soil singly. In a few instances, boron, copper, manganese, and zinc in combination gave slight to moderate increases, but of a much lower order than triple superphosphate applied to the same soil. Applied individually with phosphate they all usually increase the beneficial effect of the phosphate slightly. There are probably wheat soils in the province that will be found to give definite response to one of the trace elements, but typical browning soil is not considered to be appreciably deficient in any one of the four listed above.

7. Sulphur Treatments

In small-scale pot experiments (Table V), heavy applications of finely ground sulphur gave slight to high increases in growth of wheat seedlings in browning soil, possibly owing to its indirect action on phosphatic compounds in the soil, or to its inhibitive effects on the parasites (Table VI), as much as to its deficiency as a nutrient. Sulphur amendments usually improve root development as shown in Table VI, Soil I, by an increase in length of crown

TABLE V

EFFECT OF SULPHUR ON THE YIELD OF WHEAT SEEDLINGS IN SMALL-SCALE POT EXPERIMENTS IN THREE BROWNING ROOT-ROT SOILS

Treatment	Relative yields at six-weeks stage		
	In composite 1934 soil	*In composite* 1935 soil	*In composite* 1938 soil
Browning soil—untreated	100.0	100.0	100.0
Sulphur— 400 lb. per acre	110.9	158.1	117.8
800 lb. per acre	—	167.6	—
1000 lb. per acre	—	—	208.6
1200 lb. per acre	108.0	—	—
1600 lb. per acre	107.2	—	—
2000 lb. per acre	—	—	212.1

TABLE VI

EFFECT OF SULPHUR ON THE YIELD, LESIONING, AND DEVELOPMENT OF THE CROWN ROOTS OF WHEAT SEEDLINGS IN BROWNING ROOT-ROT SOIL

Treatment	No. of plants	Crown roots				Oven-dry weight of plants, gm.	Relative yield: Control = 100
		Length, cm.	Total no.	No. healthy	Diseased, %		
Soil I: April-May, 1934							
Browning soil—untreated	20	180	60	34	43.3	2.08	100
Sulphur—400 lb. per acre	20	227	56	40	28.5	2.60	125
800 lb. per acre	20	258	60	56	6.6	2.31	111
Soil II: February-March, 1934							
Browning soil—untreated	28	—	93	9	90.3	2.88	100
Sulphur—750 lb. per acre	28	—	122	58	52.5	3.02	104.8

roots, sometimes by an increase in number (Soil II), and by a reduction in the percentage diseased. This is accompanied by improvements in the top growth and the dry weight of the plants.

The possible effect of the sulphur in the gypsum has already been referred to. Continuous grain growing draws heavily on the soil sulphur. According to Hart and Peterson (10), cereal crops remove from the soil two-thirds as much sulphur as phosphorus. Wyatt *et al.* (22) have shown that the grey podsols of Western Canada are deficient in sulphur, but most of the black and brown soils are adequately supplied with this element. Its use as a control measure for browning root rot alone is not considered to be warranted.

Triple Superphosphate and the Response of Non-cereal Crops

The following experiments (Table VII) were conducted in an attempt to obtain more information on the relative importance of *Pythium* spp. and phosphate deficiency on browning root rot. Plants requiring a high fertility level, such as carrots and lettuce (2), were grown in (i) naturally infested

browning soil, (ii) the same soil plus triple superphosphate, and (iii) normal soil. Sweet clover (12) and buckwheat (12) which are credited with the ability to feed on certain insoluble phosphatic minerals, were used in a similar experiment. Alfalfa and flax were also included. The results with carrots and lettuce are given in Experiments 1 and 2, Table VII.

TABLE VII

THE GROWTH OF CARROTS AND LETTUCE IN INFESTED, PHOSPHATE FERTILIZED, AND NORMAL SOIL

Crop	No. of replicates	Oven-dry weight			Increase over control	
		Browning soil, gm.	Browning soil + P, gm.	Normal soil, gm.	P amendment, %	Normal soil, %
Experiment 1*						
Carrots	4	2.65	—	7.57	—	185.6
Lettuce	4	3.00	—	8.48	—	182.6
Experiment 2†						
Carrots	3	1.59	3.65	2.21	129.5	38.9
Lettuce	3	2.96	6.82	4.15	130.4	40.2

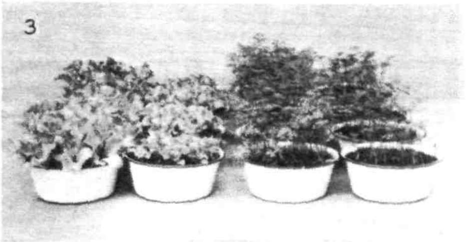
* *August 8 to October 21, 1938.*
† *October 26 to December 7, 1938.*

The carrots and the lettuce gave relatively poor growth in browning soil, good growth in normal soil, and excellent growth in browning soil to which triple superphosphate had been added (Figs. 3 and 4). The percentage increases for carrot and lettuce are virtually the same in the different tests. This indicates that the phosphate-feeding power of the two plants is similar.

For the other crops the percentage increases in air-dry weight due to the addition of triple superphosphate to browning soil were as follows: alfalfa, 41.2; buckwheat, 28.5; flax, 102.8; and sweet clover, 184.6. These experiments supply the information for which they were conducted, namely, to show whether browning soil was deficient in phosphate for non-cereal crops. For precise information on the relative response of these various crops to phosphatic fertilizers, more comprehensive tests would have to be conducted.

The roots of all these plants were free from any conspicuous lesioning such as is found on wheat seedlings grown in the same infested soil, and could be considered normal and healthy. *Pythium de Baryanum* Hesse was isolated in some instances by plating out some slightly discoloured rootlets of the carrot, lettuce, alfalfa, and sweet clover plants. In no instance was a species of *Pythium* commonly parasitic on cereals obtained. As the lesioning on the root systems of these plants was negligible, the low yield in the browning soil would appear to be due to phosphate deficiency.

With wheat, phosphate deficiency doubtless accounts for a part of the low yield in browning areas in the fallow crop, but two facts still point to the



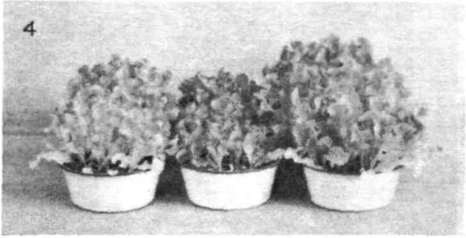


FIG. 3. *Lettuce and carrots grown in soil from normal and from browning root-rot areas of wheat fields. Normal soil is on the left and browning soil on the right of each series.*

FIG. 4. *The effect of triple superphosphate on the growth of lettuce in browning soil. Normal soil at the left; browning soil in the middle; and browning soil plus triple superphosphate at the right.*

part played by parasitic *Pythium* spp., firstly, the invariable presence of severe *Pythium* root lesioning in seedlings from such areas, and secondly, the almost universal absence of any conspicuous browning root rot in wheat on stubble, where phosphate is as low as during the fallow crop. Results reported in Part IV (20) on soil sterilization effects, indicated that root pathogens were effective in inhibiting growth and that the inhibition in browning soils could not be attributed to nutrient deficiencies alone.

Organic Amendments and Wheat Response

Improvement in the growth of wheat in browning soil from applications of cereal straw (20) suggested that information be obtained on the organic matter content of browning and normal soils. Further, as Caldwell *et al.* (4) have shown, both the nitrogen and organic matter contents of the cultivated prairie soils are being reduced by the grain and fallow system generally followed. The total nitrogen and organic carbon contents of ten paired soil samples from browning and normal areas of wheat on summer-fallowed land

have been determined, and the results are presented in Table VIII. The nitrogen was determined by the Kjeldahl procedure, and the carbon by the Walkley-Black dichromate method (21).

TABLE VIII

THE PERCENTAGES OF NITROGEN AND ORGANIC CARBON IN PAIRED SAMPLES OF BROWNING ROOT-ROT AND NORMAL SOILS*

Sample location	Total nitrogen		Organic carbon		C/N	
	Browning, %	Normal, %	Browning, %	Normal, %	Browning	Normal
1937:						
Prudhomme	.458	.460	4.11	4.10	8.97	8.91
Cudworth	.522	.543	4.63	4.65	8.87	8.51
Leofnard	.227	.206	2.45	2.15	10.80	10.44
Wakaw	.346	.369	3.14	3.22	9.08	8.73
?	.473	.269	4.26	2.47	9.00	9.18
St. Gregor	.409	.378	3.63	3.45	8.88	9.13
Engelfeldt	.471	.391	3.93	3.49	8.35	8.93
Plunkett	.267	.584	2.58	2.94	9.66	5.04
Marcelin	.389	.352	3.54	3.32	9.16	9.44
Wakaw	.447	.452	3.66	3.58	8.20	7.92
Mean	.400	.400	3.59	3.33	9.10	8.62

* *Thanks are due to G. Dion, formerly of the Soils Department, for making these determinations.*

It appears from Table VIII that there are no consistent differences in the total nitrogen, organic carbon, or C/N ratio, of browning and normal soils. The mean C/N ratio, however, is higher in the browning soils. Although there is no difference in the total nitrogen of the two soils, the nitrate nitrogen is usually slightly higher in the browning soil (Table IV, and ref. 20) at the time the disease appears.

The effects of farm manure and cereal straw. In this experiment, browning root-rot soil was filled into nine six-inch pots: three were left untreated; to another three finely divided manure was thoroughly incorporated at the rate of 15 tons per acre, and to the remaining three, chipped cereal straw at 5 tons per acre. All series were kept moist and after 10 weeks were sown to Marquis wheat. The experiment was terminated after five weeks when the control series showed severe browning symptoms. In the 30 control plants, 48 leaves were brown or yellow; in the manure series 16 were similarly discoloured, and in the straw series 26.

From the results in Table IX, it appears that the improved growth resulting from both manure and straw amendments is correlated with increased root development in which there is a higher number of roots per plant functioning in a healthy manner, and not with the percentage diseased. The washed root systems of the treated series were, however, whiter in mass than those of the controls. Improved growth is reflected in increased dry weight and a reduction in above-ground browning symptoms. These results are in confirmation of those reported earlier (20).

TABLE IX

THE EFFECTS OF MANURE AND STRAW ON THE GROWTH OF WHEAT IN NATURALLY INFESTED FIELD SOIL

January 1 to February 10, 1938

Treatment	Height, cm.	Seminal roots per plant	Crown roots			Dried plants	
			Total no.	No. healthy	Diseased, %	Weight, gm.	Increase, %
None—control	37	5.26	88	41	53.4	5.6	—
Manure	41	6.03	130	48	63.1	10.0	78.5
Straw—cereal	41	5.86	98	52	46.9	7.8	39.3

The effects of various crop residues. Freshly collected browning root-rot soil was mixed and filled into 36 one-gallon crocks sufficient for four replicates to each treatment. Farm manure at the rate of 15 tons per acre, and chipped straw, ground weed hay, and ground sweet clover hay at 3 tons per acre, were incorporated singly into the top four inches of soil of their respective series. At the same time four crocks each were sown to sweet clover and brome grass. All crocks, including 12 untreated, were kept moist. After two and a half months the sweet clover and brome plants, with as much root system as possible, were removed, ground up, and mixed into the top four inches of soil as already described. After another month all series were sown to wheat. Triple superphosphate at 200 lb. per acre and cereal straw ashes at 1000 lb. per acre were applied at the time of sowing to their respective series.

Increases in growth in the earthenware crocks follow the same trends as in pots, but are of a lower order (Table X). This is probably due to the greater room for root development, and the more compact soil with its con-

TABLE X

THE EFFECT OF ORGANIC MATTER ON THE GROWTH OF WHEAT IN BROWNING SOIL

October 25 to December 6, 1938

Treatment	Available P, p.p.m.	NO_3, p.p.m.	P/N	Crown roots			Dried plants	
				Total no.	No. healthy	Diseased, %	Weight, gm.	Increase, %
Untreated—control	42	83.3	0.50	99	61	38.3	12.9	—
Triple superphosphate	—	—	—	137	100	27.0	19.4	50.4
Farm manure	92	53.4	1.72	117	77	34.1	17.2	33.3
Cereal straw, chipped	49	41.8	1.17	76	62	18.4	14.2	10.1
Cereal straw ashes	—	—	—	128	90	29.7	15.0	16.3
Weed hay (ground)	58	100.0	0.58	111	82	26.1	15.7	21.7
Sweet clover hay (ground)	55	111.2	0.49	103	65	36.9	14.9	15.5
Sweet clover (green manure)	46	52.8	0.87	100	81	19.0	13.9	7.7
Brome grass (turned under)	52	44.8	1.16	90	76	15.0	12.9	0.0

sequent lower aeration in the crocks, both of which are known to inhibit browning root rot slightly. With the green manures of sweet clover and brome grass it is probable that insufficient time was allowed for suitable decomposition of the large amounts of fibrous material added, resulting in a deficiency of nitrogen throughout the growth period. However, with the exception of the sweet clover and brome all the other organic amendments produced a slightly beneficial carry-over effect on a second six-weeks' "wheat crop" grown in the same soil.

The slightly beneficial effect of cereal straw ashes suggests that the observed improvement from burning the stubble in browning fields is due more to the addition to the soil of the plant nutrients, especially phosphorus, which the ashes contain, than to the destruction of the parasitic fungi in the stubble. The fact that *Pythium* attacks the roots primarily, and the crowns seldom, adds further support to this view.

The available phosphorus and nitrate determinations were made at the time of sowing, but as they were determined for this single experiment only they are merely suggestive. It appears that the yield bears some relation to the phosphorus content, and little or none to the nitrate content. Owing especially to the nitrate fluctuations occurring daily in soils in pot-culture work, determinations should have been made at frequent intervals throughout the experiment.

Results to date show a constant moderate beneficial effect on wheat growth from organic matter added to browning soil.

Discussion

Of the mineral elements, phosphorus is generally recognized to be in greatest deficiency in the wheat soils of Western Canada. The rapid depletion of phosphorus in a grain system of farming is due mainly to the fact that cereal grains, which are sold off the farm, contain, according to Donaldson (7), about 75 to 85% of their total phosphorus in the seed, while phosphorus is in greater proportion in the heads than nitrogen, potassium, calcium, or sulphur. Further, as Pierre (15) has pointed out, phosphorus is found chiefly in the finest soil particles and much of it must therefore be lost by wind erosion or soil drifting. Browning root-rot soils are usually deficient in phosphorus for both cereal and non-cereal crops.

Caldwell *et al.* (4) have shown that the effects of a straight grain and fallow system on the prairie soils have been to decrease the amounts of nitrogen from 18 to 35% of the original nitrogen content as measured by the virgin sod, and to decrease the organic matter from 21 to 42% of the original organic matter content. The percentage losses increased according to soil type in ascending order as follows: black, dark brown, brown, and grey soils. The carbon was lost from the soil at a greater rate than the nitrogen. It is not surprising, therefore, that under certain conditions both nitrogenous fertilizers and organic amendments improve the growth of wheat in browning root-rot soils.

Pythium root rot is not restricted to any particular soil type, but wherever it occurs the available phosphorus is usually lower than in adjoining healthy areas. According to Russell (17) phosphate deficiency is not usually associated with any particular soil type. Also, Pierre (15) contends that it is the treatment that the soils receive after being brought under cultivation, and not the soil type, which determines the extent of phosphorus deficiency in many cases. Of all the environmental factors, phosphorus deficiency is the one most frequently associated with the increased incidence of the disease. Triple superphosphate and ammonium phosphate fertilizers, because of the production of more vigorous seedlings with a larger number of quicker growing and longer roots, give adequate control of the disease.

As recorded in the fertilizer experiments, nitrogenous compounds alone had little effect in infested soil, but once phosphate was added, further additions of nitrogen gave increases considerably higher than phosphate alone. Whereas earlier results gave no indication of any lack of nitrogen in browning soils, and indeed sometimes showed a decrease in yield when this element was applied singly, the results of the nitrogen-phosphorus combination fertilizers show that nitrogen is slightly deficient, but this deficiency is not evident as long as phosphorus is limiting. The hypothesis suggested in an earlier communication (20), that a high nitrogen-phosphorus ratio renders the plants more liable to fungal attack, would appear to be true only under conditions of low phosphorus availability. Present evidence points to the deficiency of available phosphorus as being the most important single environmental factor in the browning root-rot complex.

The work of others on the mineral nutrition of wheat may have some bearing on these results. Thus Donaldson (7) has shown that nitrate ordinarily decreases the absorption of phosphate, but that when ample phosphate is available, nitrates do not exert a depressing effect during the early periods of rapid assimilation. It is interesting to note that Carpenter (5) in Hawaii, and Le Beau (11) in the southern United States, find that nitrate applications alone increase *Pythium* root rot of sugarcane and that phosphate treatments reduce it.

The beneficial effects derived from the burning of straw are apparently due to the phosphorus which is returned to the soil and not to the destruction of the parasites in the stubble. The benefits from working in the straw are probably due to its phosphorus content as well as to stimulated biological action resulting from the fresh organic matter. The more lasting effect of turning in the straw residues, its improvement of the physical condition of the soil and its value in reducing soil erosion, make that practice more desirable than burning.

Farm manure improves seedling growth in a manner similar to that of the phosphatic fertilizers.

There is no consistent difference in the total organic matter content of browning and normal soils in the same field. Stephenson (19) has ably

emphasized the value of fresh organic matter renewal, rather than the humus level in the management of soils, and Garrett (8) has pointed out the greater effectiveness of fresh organic residues over inert humus in controlling the "take-all" disease of wheat. Several ways in which the fresh organic amendments inhibit browning root rot suggest themselves. They may result in stimulated biological activities of the soil saprophytes which would antagonize *Pythium* spp. during the active vegetative phase, or liberate nutrients from the organic matter itself, or from previously unavailable compounds in the soil. It is well known that organic materials increase the soluble phosphorus in the soil. Oberholzer (14) and Rhoades (16) in recent work incline to the view that virtually all increase in soluble phosphorus is derived from the phosphorus contained in the added material. It appears from the work of Meyer (13) that organic matter renewals are necessary for the maintenance of a good available supply of phosphorus in the soil.

The organic residues improve soil structure and reduce wind erosion, the probable deleterious effects of which on the browning root-rot situation have already been mentioned. The legumes and other non-cereal crops are primarily of value in so far as they improve soil conditions and fertility. The longevity and resistant nature of the resting spores of the parasites lessen materially the rotation value of the immunity of such plants.

These studies, together with the chemical analyses reported previously (20, Part III) on soil and plants from diseased and normal areas, suggest that browning root-rot soils have reached a point where the liberation of phosphorus and other nutrients is inadequate for crop needs. Lack of nutrient balance, as well as depletion in soil fertility, is involved. Under these conditions, the wheat seedlings are more readily attacked by several root-infecting species of *Pythium* which cannot be eradicated. It is suggested that the best means of rectifying the situation at present is by supplying supplemental nutrients in the form of artificial fertilizers, which may serve to stimulate greater biological action, and by the regular addition of organic residues or manure, which both increase nutrients and build up microbiological activities.

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A STUDY OF THE RELATION BETWEEN THE SEEDLING AND MATURE-PLANT REACTION TO *PUCCINIA GRAMINIS TRITICI* IN DURUM WHEAT CROSSES INVOLVING IUMILLO[1]

By W. H. Waddell[2]

Abstract

The seedling reaction to *Puccinia graminis Tritici*, race 21, in the greenhouse was compared with the mature-plant reaction to all races present in the field, in three intra-durum crosses involving Iumillo.

All lines that were resistant in the greenhouse were also resistant in the field. A large number of lines were susceptible at both stages. A third group contained lines susceptible in the seedling stage but resistant in the mature-plant stage. However, increasing susceptibility in the seedling stage, in general, indicated increasing susceptibility in the mature-plant stage. Most of the lines highly resistant at the mature-plant stage could have been selected on the basis of the seedling test.

Iumillo appears to possess a factor or factors for mature-plant resistance as well as a factor or factors for resistance at both stages of maturity. The reaction to stem rust in the hybrid lines did not appear to be inherited in a simple Mendelian manner. This appeared to be due, in Iumillo × Mindum crosses, to the presence of the two types of resistance in Iumillo, while in the Pentad × Iumillo crosses the inheritance was complicated still further by the additional factors for resistance in the Pentad parent.

The inheritance of seed colour was studied in a cross, Iumillo × Mindum. There appeared to be no correlation between seed colour and stem rust resistance. Seed colour in this cross was inherited in a simple 3 : 1 ratio, red-coloured seed being dominant.

The results of this study indicate that simple and inexpensive greenhouse tests, in which but one physiologic race is used, may be employed to eliminate susceptible lines in the field in durum crosses involving Iumillo.

Introduction

The extent to which a wheat cross may be tested in the greenhouse for reaction to stem rust, in order to eliminate lines susceptible in the field, is of considerable importance to the wheat breeder, especially at stations where it is difficult to obtain an adequate field epidemic.

Less labour is involved in testing the reaction of varieties to stem rust in the greenhouse than in the field. When several thousand lines are to be tested, a great deal of time and labour could be saved if simple but adequate greenhouse experiments, in which but one physiologic race is used, could be substituted for field tests.

The relation between seedling and mature-plant reaction to black stem rust in *Triticum vulgare* has received considerable attention. It has been established that the reaction to certain physiologic races in the seedling stage is not neces-

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sarily correlated with the mature-plant reaction to a number of races. Hayes and Aamodt (5) studied the inheritance of resistance to stem rust in a Marquis × Kota cross. The results indicated that certain strains were moderately resistant in the field, even though in the seedling stage they were susceptible to certain physiologic races.

Hayes, Stakman, and Aamodt (6) studied the relation between the seedling reaction and the mature-plant reaction to race 21 in the cross Marquis × Iumillo. Certain classes were obtained that were susceptible in the seedling stage to race 21 and resistant in the mature-plant stage to a number of races in the field. The Iumillo type of field resistance was found to be conditioned by at least two main factors, although modifying factors were also apparently involved.

Goulden, Neatby, and Welsh (1, 2) found that the high degree of mature-plant resistance in the strain H-44-24 was inherited entirely independently of the seedling reaction to several physiologic races. Neatby (7) reported that there was a high correlation between seedling and field reaction in the crosses Marquillo × Reward, Garnet × Marquillo, and Garnet × Double Cross. He concluded that the inheritance of the field reaction in these crosses is mainly, if not entirely, controlled by the factors that govern inheritance of the seedling reaction to race 21 in the greenhouse.

Harrington (4) studied the relation between seedling and mature-plant reaction in *T. durum*, the crosses used in this study being Kubanka No. 8 × Pentad and Mindum × Pentad. Physiologic races 1, 21, and 34 were used in the seedling studies.

The parental varieties Mindum and Pentad were tested to single races in the field by placing muslin cages over the plots, and Mindum gave the same reaction to these races in the mature-plant stage as in the seedling stage, being susceptible to both races at both stages. Pentad, on the other hand, exhibited the "mature-plant" type of resistance as reported by Goulden, Newton, and Brown (3).

Seedling reaction in the two crosses when tested with races 1, 21, and 34 appeared to depend on at least a two-factor difference. Results obtained in the Mindum × Pentad cross indicated the presence of more than one genetic factor for mature-plant reaction in the field.

In the Mindum × Pentad cross, Harrington reported a negative correlation between the seedling reaction to race 1 and the mature-plant reaction to several races in the field. No correlation was found between reaction to rust under nursery conditions and reaction to races 21 and 34 in the greenhouse in this same cross. In the Kubanka No. 8 × Pentad cross, there was no correlation between the mature-plant reaction in the field and the seedling reaction to race 34.

The durum variety Iumillo is highly resistant to stem rust, the strain used in this study being considered immune. The most extensive use of this variety in a wheat breeding program has probably been made at the Minnesota station

where a strain of Iumillo entered into the variety Marquillo and into the double cross, Thatcher.

Breeding work in which Iumillo is being used in durum crosses is under way at the Dominion Rust Research Laboratory, Winnipeg. Neatby concluded from unpublished data that there was an indication of a relation between the field reaction and the reaction in the greenhouse to physiologic race 21 in durum crosses in which Iumillo was one of the parents. The results of a further investigation of this possibility are given in this paper.

Materials and Methods

The inheritance of reaction of Iumillo to stem rust was studied in three durum wheat crosses. The first two were Iumillo × Mindum "A" and Iumillo × Mindum "B", the third being a Pentad × Iumillo cross.

Iumillo is a durum wheat of Italian origin. The strain used is immune from rust in both seedling and mature-plant stages. It is a red-seeded variety and is not of commercial importance, since the macaroni produced from it is of inferior quality.

Pentad is a durum wheat introduced from Russia by Dr. R. L. Bolley of the North Dakota Agricultural Experiment Station. It is usually resistant, having from 5 to 10% of stem rust. It is a high yielding variety, but, like Iumillo, is low in quality.

Mindum, as the name implies, was produced at the Minnesota station, University Farm. It is an amber durum selection of high macaroni quality and is moderately susceptible to stem rust.

Seedling tests of the hybrid lines and parents were made by planting about 15 seeds of each line in 4-inch pots and inoculating with race 21. Duplicate tests were made in all cases and the two results were combined. The type of pustules obtained is illustrated in Fig. 1. Classes $X^{=}$ and X^{+} were not included, since the slight difference between these classes and the adjacent ones would not be noticeable in a photograph.

Seedling reaction to race 21 was found to be mainly of the X type. This type of reaction was first described by Stakman and Levine (8) and is a common one in durum wheats. In the X reaction, several types of infection are to be found on the same leaf. These may range from the highly resistant 0; (zero, fleck) type to the susceptible 4 type. The reaction in the Iumillo × Mindum crosses was recorded as follows: 0;, $X^{=}_{-}$, X^{-}, X, X^{+}, X^{+}_{+}, and 4. In the Pentad cross, an additional class was observed. This was class $3^{\pm}$, which is the seedling reaction of the Pentad parent.

Field reaction to a number of races in the cross Iumillo × Mindum "A" was recorded in percentage of infection, in classes of 10%, from 0 to 90. An additional class, trace (1%) was used in the other crosses.

The reaction of the parental varieties, both in seedling and mature-plant stage, is given in Table I.

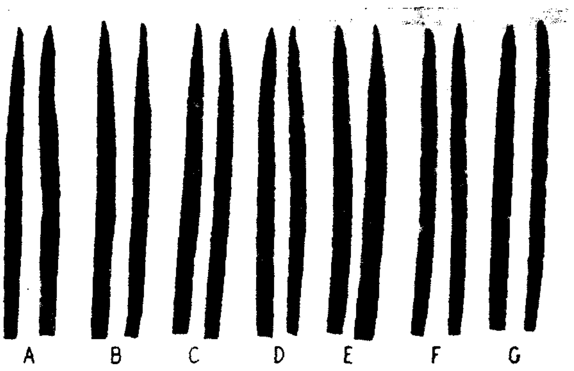

FIG. 1. *Reaction of Mindum and Iumillo parents and F_3 families in the seedling stage to physiologic race 21 in the greenhouse.* A = *Mindum*, B = *4*, C = $X^{=}$, D = X, E = X^{+}, F = *0;*, G = *Iumillo*.

TABLE 1

REACTION OF PARENTAL VARIETIES IN THE SEEDLING STAGE TO RACE 21 IN THE GREENHOUSE, AND IN MATURE PLANTS TO A COLLECTION OF RACES IN THE FIELD

Parents	Seedling reaction class	Percentage of infection of mature plants
Iumillo	0;	0
Pentad	3:	20*
Mindum	4	40–50

* *This reaction is unusually high for Pentad.*

The mean reaction in the seedling tests was calculated by assigning the following numerical values to the different classes in both of the Iumillo × Mindum crosses:

Numerical value	1	2	3	4	5	6
Seedling reaction	0;	$X^{=}$	X^{-}	X	X^{+}	X^{++} and 4

There was little, if any, difference between the field reaction of the two seedling classes X^{++} and 4. These two classes were, therefore, given the same numerical value when computing the means.

In computing the means for the percentage of infection in the field in the crosses Iumillo × Mindum "B" and Pentad × Iumillo, the following numerical values were assigned:

Numerical value	1	2	3	4	5	6	7	8	9	10	11
Percentage of infection in the field	0	1	10	20	30	40	50	60	70	80	90

The class 1% contained only plants with a slight trace of stem rust. These data show that Iumillo has an immune type of reaction to stem rust both in seedling and mature plant stage. Mindum was susceptible in the seedling stage and had 40 to 50% rust in the mature-plant stage. Pentad was slightly less susceptible in the seedling stage to race 21. This variety had a $3^{\pm}$ type of infection and showed more resistance than Mindum in the mature-plant stage.

Experimental Results

Iumillo × Mindum "A"

The first study comprised 125 plants from F_3 lines of an Iumillo × Mindum cross. These lines had been grown in the field in 1934, and notes on the mature-plant reaction to rust were obtained in classes from 0 to 90%.

These data were available at the commencement of the present study and the material was used to obtain some preliminary evidence as to the relation between seedling and mature-plant reaction to stem rust in this cross.

Approximately 15 seeds from each plant were sown in pots in the greenhouse at University Farm, St. Paul, Minnesota, in March 1936. These were inoculated with race 21 and notes taken on the basis of all plants in the pot. The basis of classification was as outlined previously.

A duplicate test of seedling reaction to race 21, from seed of the same 125 plants, was made in the greenhouse at the Dominion Rust Research Laboratory Winnipeg, in April, 1936, and the reaction determined on the same basis of classification as at University Farm. Table II shows the close relation between the two independent tests.

TABLE II

Correlation between the seedling reaction to race 21 in the cross Iumillo × Mindum "A" as determined in tests at University Farm and Winnipeg

		University Farm, St. Paul						Total
		0;	$X^{=}$	X^{-}	X	X^{+}	X^{++} and 4	
Dominion Rust Research Laboratory, Winnipeg	0;	4		1				5
	$X^{=}$	8		2				10
	X^{-}	1	1	5	3	1		11
	X		1	3	7	10	4	25
	X^{+}			5	3	4	10	22
	X^{++} and 4				4	8	40	52
	Total	13	2	16	17	23	54	125

A correlation coefficient of +0.82 was obtained. Only 17 plants out of a total of 125 differed by more than one class in the two independent experiments. This is a fact of considerable interest, since it shows that although the reactions dealt with were mainly of the X type, which is usually considered rather variable, a very close agreement was obtained even though the duplicate tests were made in different greenhouses at different times and under different conditions of light and temperature. It might be expected that, had the duplicate tests been made under the same conditions, an even higher correlation might have been obtained. This indicates that duplicate seedling tests can be made, with very satisfactory results, over a relatively wide variety of conditions in the Iumillo × Mindum cross.

A comparison between the F_4 seedling reaction to race 21 and the F_3 mature-plant reaction to a number of races in the field in this cross is presented in Table III. The data in this table show that a certain amount of association exists between the two reactions.

TABLE III

DISTRIBUTION OF THE MEANS OF THE F_3 LINES OF THE CROSS IUMILLO × MINDUM "A" FOR THE REACTION TO RACE 21 IN THE SEEDLING STAGE IN THE GREENHOUSE, AND THE MATURE-PLANT REACTION IN THE FIELD

Mean F_4 mature-plant reaction	Mean F_4 seedling reaction											Total
	1.0	1.1–1.5	1.6–2.0	2.1–2.5	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0	5.1–5.5	5.6–6.0	
1	4	6	3	3	5	1	1		1		1	25
3		2			1	3	2	2	1	1		12
4						1	3	2	2	3	3	14
5						1	2	1				4
6							2	3				5
7								2		4	6	12
8							1	2	3	2	12	20
9							1		4	2	13	20
10									3	2	6	11
11										2		2
Total	4	8	3	3	6	6	12	12	14	16	41	125

Lines that were highly resistant in the seedling stage were also resistant in the mature-plant stage. A large number of lines were susceptible at both stages. However, there appeared to be a third group which was susceptible in the seedling stage but showed considerable resistance in the field. Therefore, a high correlation between the reactions at the two stages was not obtained.

IUMILLO × MINDUM "B"

In the second cross of Iumillo × Mindum "B", 303 F_3 lines were studied for reaction to stem rust. These lines were a random sample selected from the F_2 plots grown in the field in the summer of 1935 at the Dominion Rust Research Laboratory, Winnipeg, and were made available to the writer through the kindness of Dr. C. H. Goulden.

The F_2 plants were threshed and the seed divided into two lots, one for a seedling test to race 21 in the greenhouse and the remainder for a test in the mature-plant stage in the field. Duplicate plantings were made for each line in the greenhouse, the same technique being used as for Iumillo × Mindum "A". An average of about 20 seedlings of each of the 303 F_3 lines were tested in the greenhouse. The results were recorded, the scale outlined previously being used.

During the summer of 1936, the reserve seed of the 303 F_2 plants of Iumillo × Mindum "B" was planted in one-row plots in the field, in order to study the F_3 mature-plant reaction. The rows were 5 ft. long and about 25 seeds were spaced uniformly in each row.

In the fall, these one-row plots were pulled separately and notes on rust reaction were taken on each individual plant within the lines. This method would be expected to give a better estimate of the genetic composition of the F_2 plants than had the readings been made on the 303 F_2 plants themselves. This permitted a study of the association of greenhouse and field reactions based on the same generation. The basis of classification for the mature-plant reaction was the same as in the Iumillo × Mindum "B" cross, with the exception that an additional class trace (1%) was used.

The natural rust epidemic in the field during the summer of 1936 was not heavy. As, however, inoculum was placed in the field, a fairly heavy artificial epidemic was obtained. That the epidemic was not so severe as is sometimes obtained is indicated by the fact that no plants were classified as having more than 70% of stem rust. In the case of Iumillo × Mindum "A", readings as high as 90% were obtained. The Mindum parent had from 40 to 50% of rust. The strain of Mindum used in this study is one that had been selected for susceptibility as a differential host. The most prevalent races in 1936 were 21, 36, and 56.

The reactions of the parental varieties and of the F_3 lines in the field at the Dominion Rust Research Laboratory, Winnipeg, in the summer of 1936 are given in Table IV.

TABLE IV

DISTRIBUTION OF THE MEANS FOR MATURE-PLANT REACTION OF THE PARENTS AND F_3 LINES OF THE CROSS IUMILLO × MINDUM "B"

Parent or F_3 lines	Mean F_3 field reaction																	Total
	1.0	1.1–1.5	1.6–2.0	2.1–2.5	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0	5.1–5.5	5.6–6.0	6.1–6.5	6.6–7.0	7.1–7.5	7.6–8.0	8.1–8.5	8.6–9.0	
Iumillo	67																	67
Mindum													20		22			42
F_3	7	11	9	19	27	23	30	27	28	23	21	20	17	19	11	8	3	303

The distribution of the F_3 is continuous from the reaction of the Iumillo to the reaction of the Mindum parent, even exceeding the latter in susceptibility. This would indicate that the mode of inheritance or reaction to rust

in the mature-plant stage is governed by more than one factor. It is noted that seven F_3 lines were obtained that had only immune plants like the Iumillo parent, and 11 lines that had only a trace of stem rust. From the practical standpoint, it appears that rust-resistant lines can be obtained without much difficulty.

The reaction in the seedling stage to race 21 of the 303 F_3 lines of the cross Iumillo × Mindum "B" is shown in Table V.

TABLE V

DISTRIBUTION OF THE MEANS FOR SEEDLING REACTION TO RACE 21 OF THE PARENTS AND OF THE F_3 LINES IN THE CROSS IUMILLO × MINDUM "B"

Parent or F_3 lines	Mean F_3 seedling reaction											Total
	1.0	1.1–1.5	1.6–2.0	2.1–2.5	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0	5.1–5.5	5.6–6.0	
Iumillo	120											120
Mindum											132	132
F_3	8	4	5	10	8	18	20	31	35	48	116	303

The distribution of the F_3 lines indicates that the seedling reaction to race 21 in this cross is controlled by more than one genetic factor.

The relation between mean percentage rust infection in the field and mean pustule type of seedling infection in the greenhouse is shown in Table VI.

TABLE VI

DISTRIBUTION OF THE MEANS OF THE F_3 LINES OF THE CROSS IUMILLO × MINDUM "B" FOR THE REACTION TO RACE 21 IN THE SEEDLING STAGE IN THE GREENHOUSE, AND THE MATURE-PLANT REACTION IN THE FIELD

Mean F_3 mature-plant reaction	Mean F_3 seedling reaction											Total
	1.0	1.1–1.5	1.6–2.0	2.1–2.5	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0	5.1–5.5	5.6–6.0	
1.0	7											7
1.1–1.5	1	3	4	3								11
1.6–2.0		1		1	2	3		1	1			9
2.1–2.5			1	3	1	2	2	4		5	1	19
2.6–3.0				1	1	6	4	3	6	1	5	27
3.1–3.5					1	2	4	2	2	5	7	23
3.6–4.0				2	2	1	2	3	7	6	7	30
4.1–4.5					1	2	4	5	4	6	5	27
4.6–5.0						1	1	6	5	6	9	28
5.1–5.5							1	4	3	6	9	23
5.6–6.0						1	1	1	2	3	13	21
6.1–6.5							1	2	4	4	9	20
6.6–7.0									1	3	13	17
7.1–7.5										1	18	19
7.6–8.0										1	10	11
8.1–8.5										1	7	8
8.6–9.0											3	3
Total	8	4	5	10	8	18	20	31	35	48	116	303

As in the case of Iumillo × Mindum "A", a high correlation between the reactions at the two stages was not obtained. However, all lines that were highly resistant to race 21 in the seedling stage were resistant in the field when inoculated with a collection of races, and a large number of lines were obtained that were susceptible at both stages. Some lines that were susceptible in the greenhouse to race 21 were resistant in the field. It may be observed, however, that as susceptibility increased in the seedling stage, there was an increase in the amount of rust found on the lines in the mature-plant stage. Seven of the 303 F_3 lines were completely free from rust, both in the seedling and in the mature-plant stage. This would indicate that, for plant breeding purposes, lines that in the seedling stage showed high resistance to race 21 would be resistant in the mature-plant stage to a number of races.

The standard deviation for field reaction of each of the 303 lines was calculated separately. The association between means and standard deviations is given in Table VII.

TABLE VII

RELATION BETWEEN MEAN AND STANDARD DEVIATION OF FIELD RUST REACTION OF F_3 LINES IN THE CROSS IUMILLO × MINDUM "B"

Mean rust classes	Standard deviation													Total
	0.0–0.25	0.26–0.50	0.51–0.75	0.76–1.00	1.01–1.25	1.26–1.50	1.51–1.75	1.76–2.00	2.01–2.25	2.26–2.50	2.51–2.75	2.76–3.00	3.01–3.25	
1.0	7													7
1.1–1.5		6	2	2	1									11
1.6–2.0				5	2	2								9
2.1–2.5		3	1	3	2	6	2	1	1					19
2.6–3.0		1	1	8	6	1	1	5	2	2				27
3.1–3.5			1		3	2	3	4	8	1	1			23
3.6–4.0					1	2	4	4	10	5	3	1		30
4.1–4.5						4	5	2	5	2	5	2	2	27
4.6–5.0					3		4	8	5	2	3	2	1	28
5.1–5.5				2	3	4	8		2	2	1	1		23
5.6–6.0			1	3	4	3	3	2	1	2	1		1	21
6.1–6.5		1	3	4	4	1	3		1	3		2		20
6.6–7.0		1	4	4	5		2			1				17
7.1–7.5	1	3	6	2	3		1	1	1	1				19
7.6–8.0	2	1	2	3	2			1						11
8.1–8.5	1	5	1	1										8
8.6–9.0	3													3
Total	14	21	22	36	38	25	36	28	36	21	14	8	4	303

It is of some interest to find that no F_3 lines with an intermediate mean reaction to rust had low standard deviations. This group exhibited greater variation between plants within a line than did the more resistant or susceptible lines. These data indicate that, in this intermediate group, there were no F_3 lines that were breeding true for reaction to rust.

Mature-plant Resistance Study

In order to test further the mature-plant resistance of Iumillo, certain lines of the cross Iumillo × Mindum "B" were studied. The mature-plant reaction of 34 F_3 lines was compared with the seedling reaction in these same lines to race 21 in the F_3 and F_4 generations.

Eighty-five plants were selected in the field from 34 F_3 lines and these plants were tested in the seedling stage in the F_4 generation to race 21. These plants were chosen mainly from the seedling susceptible-mature plant resistant group in the F_3 population, the purpose being to ascertain whether these lines that had been susceptible in the F_3 seedling stage and resistant in the F_3 mature-plant stage would show susceptibility in the F_4 seedling test. A few plants were tested also from each of the seedling resistant-mature plant resistant and seedling susceptible-mature plant susceptible groups to test the stability of these reactions.

Comparisons were made between the F_3 mature-plant reaction and the reaction in the F_3 and F_4 seedling stage. The data obtained from these comparisons are shown in Tables VIII and IX. In Table VIII, the field reaction of the single plant selections is compared with the mean seedling reaction of the line from which the plant was selected. In Table IX the mature-plant reaction of the F_3 plant selections is compared with the mean seedling reaction of the F_4 progeny.

TABLE VIII

COMPARISON OF MATURE-PLANT REACTION OF SELECTED F_3 PLANTS WITH MEAN SEEDLING REACTION OF THE F_3 LINES

F_3 field reaction	Mean F_3 seedling reaction											Total
	1.0	1.1–1.5	1.6–2.0	2.1–2.5	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0	5.1–5.5	5.6–6.0	
1	6	2	1	4	1	4	1	2	4	1	2	28
2		1		1	1	3	1	2	2	6	8	25
3						1		4		4	8	17
4								1		1	5	7
5								1			2	3
6									1	1	1	3
7											1	1
8										1		1
Total	6	3	1	5	2	8	2	10	7	14	27	85

The data indicate clearly that those lines that were resistant in the field but susceptible in the seedling stage in F_3 were also susceptible in the seedling stage in the F_4 generation. This is evidence that these lines possess a type of mature-plant resistance, although in the seedling stage they are susceptible. The data also show that the F_3 seedling-resistant lines transmitted their resistance to the F_4 generation and that those lines that were susceptible in both field and greenhouse in F_3 were again susceptible in the seedling stage in the F_4 generation.

TABLE IX

Comparison of mature-plant reaction of F_3 plants with mean seedling reaction of F_4 progeny

F_3 field reaction	Mean F_4 seedling reaction											Total
	1.1	1.1–1.5	1.6–2.0	2.1–2.5	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0	5.1–5.5	5.6–6.0	
1	11	3	1	1		1	1		3	2	5	28
2	1			1	1		2		1	1	18	25
3									2	1	14	17
4											7	7
5											3	3
6										1	2	3
7											1	1
8											1	1
Total	12	3	1	2	1	1	3	0	6	5	51	85

Relation of Stem Rust Resistance to Seed Colour

Seed colour is an important factor in durum wheats, since only white-seeded varieties are suitable for the production of high quality macaroni.

Notes were taken on the seed colour of 152 F_2 lines of the Iumillo × Mindum "B" cross. The relation between seed colour and stem-rust reaction in the field in F_3 is given in Table X.

TABLE X

Relation between mature-plant reaction to stem rust and seed colour in a Iumillo × Mindum cross

Seed colour of F_2 plants	Reaction to stem rust in F_3 lines*					Total
	R	MR	SR	MS	S	
White	8	2	6	5	10	31
Red	31	16	9	23	42	121
Total	39	18	15	28	52	152

* R = *resistant;* MR = *moderately resistant;* SR = *slightly resistant;* MS = *moderately susceptible;* S = *susceptible.*

The distribution given in Table X indicates that there was no association between seed colour and stem-rust reaction in this cross. A ratio of 31 white to 121 red seeded plants in F_2 was obtained. This gives a good fit to a 3 : 1 ratio, the X^2 value being 1.72. It is apparent that seed colour in the Iumillo × Mindum cross is governed by a single factor pair.

Pentad × Iumillo

The third cross studied, Pentad × Iumillo, included 147 F_3 lines which constituted a random sample from the F_2 population grown at the Dominion Rust Research Laboratory, Winnipeg, in the summer of 1935.

No rust notes were taken on the F_2 plants. The F_2 plants were threshed and the seed divided, one lot being used for the seedling test and the remainder for the field test. The field test was planted in one-row plots as in the former study. Notes on the mature-plant reaction were taken on each individual plant in the lines. Mean values were computed for each F_3 line. The results are given in Table XI.

TABLE XI

Distribution of the means for mature-plant reaction of the parents and of the F_3 lines in the Pentad × Iumillo cross

Parent or F_3 lines	Mean F_3 field reaction															Total
	1.0	1.1–1.5	1.6–2.0	2.1–2.5	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0	5.1–5.5	5.6–6.0	6.1–6.5	6.6–7.0	7.1–7.5	7.6–8.0	
Iumillo	85															85
Pentad					21		38		10							67
F_3	5	31	26	25	16	6	10	6	3	5	5	3	2	2	2	147

Both parent varieties would be classed as resistant. As previously mentioned, the readings in the present study are unusually high for Pentad. The distribution of the mean reaction of the F_3 lines greatly exceeded the limit of Pentad, the less resistant of the two parents. Nineteen F_3 lines were more susceptible than the Pentad parent. Five lines were as resistant as the Iumillo parent, approximating the same ratio as obtained in the cross of Iumillo × Mindum "B". There was a preponderance of resistant F_3 lines, since both of the parents are resistant.

The relation between the mean reactions on the F_3 lines in the mature-plant stage and on the seedlings is given in Table XII.

The distribution of the F_3 lines, showing the relation between seedling and mature-plant reaction, is similar to that obtained in the cross Iumillo × Mindum "B" (see Table VI). No F_3 lines that were resistant in the seedling stage were susceptible in the field. Only two of the 147 F_3 lines were completely free from rust in both greenhouse and field tests.

If data, such as are given in Table XII, are to be used to predict rust reaction in the mature-plant stage, an arbitrary division must be made between the seedling classes. If the four classes having the lowest mean seedling reaction are selected and grown under epidemic conditions in the field, none of the plants in the 35 lines in these classes will show any appreciable degree of rust. If, in addition, the two adjacent classes are also selected for field observation, 34 additional lines will be grown and only four of these will show any con-

TABLE XII

DISTRIBUTION OF THE MEANS OF THE F_3 LINES OF THE CROSS PENTAD × IUMILLO FOR THE REACTION TO RACE 21 IN THE SEEDLING STAGE IN THE GREENHOUSE, AND THE MATURE-PLANT REACTION IN THE FIELD

Mean F_3 mature-plant reaction	Mean F_3 seedling reaction*											Total
	1.0	1.0–1.5	1.6–2.0	2.1–2.5	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0	5.1–5.5	5.6–6.0	
1.0	2	3										5
1.1–1.5		2	3	9	5	2	3	6		1		31
1.6–2.0		1	3	8	3	4	3	1	2	1		26
2.1–2.5		1			6	6	3	7	1		1	25
2.6–3.0		1	2		2	2	4	2	1	2		16
3.1–3.5					1	1		3			1	6
3.6–4.0						1	5	2	1		1	10
4.1–4.5						1		3		2		6
4.6–5.0								1	1	1		3
5.1–5.5								2	1	2		5
5.6–6.0							1	2	1		1	5
6.1–6.5							1	1			1	3
6.6–7.0							1		1			2
7.1–7.5								1			1	2
7.6–8.0							1		1			2
Total	2	8	8	17	18	16	25	28	12	7	6	147

* *The seedling reaction class 3± was combined with the X class and given a numerical value of 4 in computing the means.*

siderable degree of rust. If this second division is made, about 38 lines that possess "mature-plant" resistance will be discarded.

It will be noted that the number of lines possessing "mature-plant" resistance in this cross is much larger, proportionally, than the similar group in the Iumillo × Mindum "B" cross. This is, no doubt, due to the additional factor or factors for "mature-plant" resistance in the Pentad parent of the Pentad × Iumillo cross.

Discussion

The elimination of susceptible lines is one of the main considerations in a wheat breeding program in which rust resistance is one of the primary objectives. Extensive field tests under artificial epidemics are laborious and expensive and at certain breeding stations are not practical. Simple greenhouse tests may be made with lower labour costs than field tests.

The results reported in this paper indicate that, in durum crosses involving Iumillo, there is a certain amount of correlation between the seedling reaction in the greenhouse and the reaction of the mature plants in the field. In each of the crosses studied, three groups were obtained. The first group was resistant at both stages and the second was susceptible at both stages. The data indicate that in these two groups the same factors control the reaction at the two stages of development.

The third group consisted of lines that were susceptible in the seedling stage but resistant as mature plants in the field. It is apparent that as the plants in these lines approach maturity a type of reaction develops that is different from that observed in the seedling stage. This is what is known as "mature-plant" resistance.

The variety Iumillo, therefore, appears to possess at least two main types of resistance. One type is similar to that reported by Neatby (7) in a study of three crosses, all of which involved Iumillo resistance. In these crosses a high correlation was found between the reaction of the seedling plants and the mature plants, and it was concluded that the field reaction was controlled, mainly, by the factors that govern the seedling reaction to race 21 in the greenhouse.

On the other hand, Iumillo appears to possess the "mature-plant" type of resistance as reported by Goulden *et al.* (1, 2). Table VI shows that about 48 out of 303 lines were susceptible in the seedling stage but were classified as resistant in the field, thereby indicating that they possess "mature-plant" resistance.

In this regard, it is interesting to note the results in the Pentad × Iumillo cross. Goulden, Newton, and Brown (3) demonstrated that Pentad possesses a type of "mature-plant" resistance similar to that of H-44-24 and Hope. If Iumillo also possesses a type of "mature-plant" resistance it would be expected that more plants would exhibit this resistance in the Pentad × Iumillo cross than in the Iumillo × Mindum cross. An examination of Tables VI and XII shows that such is the case. In the Iumillo × Mindum cross about 48 out of 303, or 15% of the lines, possessed "mature-plant" resistance, while in the Pentad × Iumillo cross about 68 out of 147, or 46% of the lines, possessed this type of resistance.

The seedling reactions in the crosses studied are mainly of the X type, which in the Iumillo × Mindum cross appears to be quite stable from one experiment to another. Any lines that receive a reading of $X^{=}$ or X^{-} will invariably be resistant in the mature-plant stage. Lines receiving a reading of X, X^{+}, or $X^{\ddagger}$ may be either weakly resistant or susceptible in the mature-plant stage. Nevertheless, the data show quite conclusively that as the readings increase from X up to $X^{\ddagger}$ there is a marked increase in susceptibility in the field.

There appears to be no indication in this study that the reaction to stem rust is inherited in a simple Mendelian manner. The distribution of the F_3 lines of the Iumillo × Mindum "B" cross for rust reaction in the field, together with the distribution obtained when the standard deviation of the means for mature-plant reaction are plotted, indicates, however, that the field reaction to stem rust in this cross is not inherited in too complex a manner to be of value from a plant breeding standpoint.

Simple inheritance in the mature-plant stage of the Pentad × Iumillo cross is not indicated by the data. Simple inheritance in the seedling stage was not indicated in any of the crosses studied.

From the standpoint of plant breeding technique, the results obtained in this study have clearly demonstrated that lines that were resistant in the seedling stage to race 21 were resistant at the mature-plant stage to all races present in the field. In a breeding program involving Iumillo × Mindum crosses, it would appear that resistance can be achieved by growing a large F_2 population and then testing the seedlings from this generation to race 21 in the greenhouse. According to the results reported in this paper, from 9 to 15% of the F_3 lines would be resistant in the seedling stage and would, therefore, be resistant as mature plants as well.

This method will eliminate some lines resistant in the mature-plant stage, as a number of lines were obtained that were susceptible in the seedling stage but resistant as mature plants in the field. However, this group did not have as high a type of field resistance as those that possessed seedling resistance also.

Unpublished data* would seem to indicate that in lines of *Vulgare* type, from crosses between Iumillo and varieties of *Triticum vulgare*, two types of resistance, similar to those described above, were present. It might be noted again that in this entire study only one physiologic race was used to determine the seedling reaction in order to forecast the reaction of the mature plants in the field.

The colour of the seed is important in durum breeding, as only amber-seeded varieties produce high quality macaroni. The results of this study indicate that in Iumillo × Mindum crosses, seed colour is dependent on a single factor pair difference, with red seed dominant. There appears to be no correlation in these crosses between rust reaction and seed colour.

Acknowledgments

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* *Unpublished data, Dr. R. F. Peterson, Dominion Rust Research Laboratory, Winnipeg Manitoba.*

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COMPARISON OF DIPLOID AND TRIPLOID SUGAR BEETS[1]

BY F. H. PETO[2] AND J. W. BOYES[2]

Abstract

In a small replicated field trial, percentage sugar and root weight were negatively correlated in both diploid and triploid individual beets, but the decreases in percentage sugar for every 100-gm. increase in root weight differed significantly, being on the average 0.34% for the diploids and 0.17% for the triploids. As root weight increased beyond 900 gm. the yield of sugar per 100 gm. of root weight increased more rapidly in the triploids than in the diploids. Under the conditions of this test, triploid beets exceeded the diploids in root weight by 12.2%, in yield of sugar per beet by 14.9%, and in dry top weight by 17.8%. The triploids exceeded the diploids in area index of the leaves by 34.4% and in area index of the stomata by 42.6%.

Induced Tetraploidy in Sugar Beets and the Origin of Triploid Progeny

Colchicine treatments of diploid sugar beet stecklings from a selection of the variety "Great Western" resulted in the production of tetraploid sectors or branches. The colchicine was dissolved in an agar gel and applied in gelatine drug capsules which had been previously waterproofed with a coating of collodion. The young shoots or branch tips were decapitated and capsules containing 0.1 or 0.5% colchicine were slipped over the cut end. The colchicine affected the axillary buds just below the point of decapitation, and by cutting back all normal shoots, growth in the affected shoots was promoted. Abnormally large pollen grains were found on certain branches of five of the 33 stecklings treated. Chromosome counts were made on 206 of the progeny of affected branches, and 69 triploids and two tetraploids were found. It was obvious that the majority of the tetraploid flowers must have been fertilized with pollen from diploid sectors of the same plant or from adjacent diploid plants.

At approximately the same time that the above triploids and tetraploids were produced, Frandsen (2), Rasmusson and Levan (10), and Schwanitz (13) were also successful in obtaining tetraploids by colchicine treatments, but the method of application differed in each case from that outlined above.

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Methods of Testing Diploid and Triploid Beets

Fifty-six triploids and the same number of diploids were obtained from seed sown in the greenhouse on January 11, 1939. They were transplanted to the field on May 29 and planted in rows of seven plants with diploid and triploid rows alternated. The rows were spaced 22 in. apart and the beets planted 10 in. apart in the rows. At the time of planting, the triploids were slightly larger than the diploids, and the difference in size became more pronounced as the season advanced. Unfortunately, very heavy rains in late July and mid-August caused temporary flooding over three-quarters of the experimental plots. This checked development greatly in the area affected and resulted in a very wide range in beet size. The plots were replicated in such a manner that the diploids and triploids appeared to be equally affected so that the experiment was not lost, although the conditions throughout development were by no means typical of normal commercial conditions where seed is sown directly in the field in the spring and the plants are grown under normal moisture conditions. Nevertheless, information on the relative behaviour of diploid and triploid beets under these conditions should be of value.

Leaf dimensions of five of the largest leaves on each plant were taken on August 8. A slight difficulty was encountered in determining the length of the lamina owing to differences in the form of the leaf base. However, one observer took all the measurements, and maximum length and width of the lamina were recorded.

Epidermal strips were taken from the lower surfaces of two well developed leaves of each diploid and triploid plant on August 9. These were fixed in absolute alcohol 3 parts, and acetic acid 1 part. They were later examined, and the length and total width of ten pairs of guard cells from each plant were measured.

The beets were harvested on October 4. The tops were removed just below the lowest living leaf scar, weighed immediately, and the dry weight was obtained after passing them through a forage crop drier. The roots were washed, dried, and weighed in preparation for sugar analysis.

Individual beets were analysed for sugar by hot water digestion, Method I (1). Ten cc. of lead acetate, 0.25 cc. of concentrated acetic acid, and two drops of ether were the quantities of reagents used per determination. The percentage sugar was determined by polarimetric readings obtained with a Hilger polarimeter with 200 mm. tubes and a sodium lamp. A factor of 0.7519 was used for conversion to percentage sugar and 200/26 for dilution correction, the combined factor being 5.7838.

Preliminary tests revealed the difficulty of obtaining a representative sample from the beet. This was finally overcome by dividing the root longitudinally and grinding one half in a meat chopper, using a plate with $\frac{1}{4}$-in. perforations. Before taking the 26-gm. sample, the pulp was mixed thoroughly with a spoon for approximately 90 sec. In this way it was possible

to obtain agreement between duplicate samples to within 0.1% sugar on the average, with a maximum difference of 0.2%. Duplicate samples were taken from every fifth beet and single determinations were run on the remainder.

Percentage of Sugar and Root Weight

Several investigators have studied the relation between percentage sugar and root weight. Pack (8) summarized the evidence prior to 1930 and concluded that there is, in general, a negative correlation between these two characters. Subsequent studies by Immer (4) and Garner and Sanders (3) support this general conclusion. A summary of the data by several authors as shown in Table I reveals that the correlation coefficients secured vary from about −0.2 to −0.7 and that the average percentage decrease in sugar per increase of 100 gm. in root weight varies from 0.10 to 0.39. In one instance a significant positive correlation was reported.

TABLE I

COMPARISON OF THE RESULTS OF VARIOUS AUTHORS WITH REGARD TO THE CORRELATION OF PERCENTAGE SUGAR AND ROOT WEIGHT

Author	Remarks	Average % change in sugar for increase of 100 gm. in root weight	Correlation coefficients
Rümker (11)		−0.29	
Novotný (6)		−0.39 to −0.19	
Pack (8)			−0.53 ± 0.05 −0.52 ± 0.05 −0.48 ± 0.06 −0.33 ± 0.06 −0.27 ± 0.06
Immer (4)		−0.13	−0.26
Oetken (7)		−0.10	
Garner and Sanders (3)	Spacing 12 × 6 in. Spacing 18 × 9 in.	+0.51 −0.19	+0.45* −0.34*
Peto and Boyes	Diploids Triploids	−0.34 −0.17	−0.74** −0.54**

* *Exceeds 5% level of significance.*
** *Exceeds 1% level of significance.*

Genetical differences in the strains tested may account for some of the variation noted in Table I. For example, Pack (8) studied five very different lines and found that r values ranged from −0.27 to −0.53. Under strictly comparable environmental conditions, Garner and Sanders (3) studied the effect of various spacings and revealed that environmental conditions may influence the relation between sugar and root weight. Close spacings of

12 × 6 in. during a hot dry season resulted in a correlation coefficient of +0.451 and 0.51% increase in sugar percentage for 100 gm. increase in root weight. Wider spacings of 18 × 9 in. during a more favourable season gave a correlation coefficient of −0.336 and 0.19% decrease in sugar percentage for 100 gm. increase in root weight. The authors point out that the beets giving the positive correlations were very small, averaging only 163 gm. each, which shows the extremely unfavourable environmental conditions under which they developed.

In the present investigation the correlation coefficient between percentage of sugar and weight of individual beets for the diploids is −0.74 and for the triploids is −0.54. The r value for the diploids is much higher than any other values listed in Table I, whereas the triploid r value is similar to the higher values reported by Pack (8) and Garner and Sanders (3).

TABLE II

TESTS OF SIGNIFICANCE OF DIFFERENCES IN DIPLOID AND TRIPLOID REGRESSION FUNCTIONS

Regression of	Source of variance	D.f.	Mean square
Yield of sugar on root weight	Difference in regression coefficients	2	1,976**
	Residuals	106	135
Percentage sugar on root weight	Difference in regression coefficients	1	12.47**
	Residuals	108	1.30
Yield of sugar on dry top weight	Difference in regression coefficients	2	1,621
	Difference in centroids	1	717
	Residuals	106	752
Root weight on dry top weight	Difference in regression coefficients	1	1
	Difference in centroids	1	33
	Residuals	108	38,492

** *Exceeds 1% level of significance.*

The diploids and triploids differed significantly in regard to slope of their regression lines, the differences exceeding the 1% level of significance (Table II). The average decrease in percentage sugar for every 100 gm. increase in root weight was 0.34% for the diploids and 0.17% for the triploids. The data are illustrated in Fig. 1, in which the more nearly horizontal triploid curve shows quite clearly that percentage sugar does not decrease with increased size of root as rapidly in triploid as in diploid beets. This difference cannot justifiably be ascribed to environmental differences nor to any great extent to qualitative differences in genic constitution, since the triploid originated from the diploid material. Consequently the addition of an extra set of chromosomes must have affected the slope of the line of regression of percentage sugar on root weight.

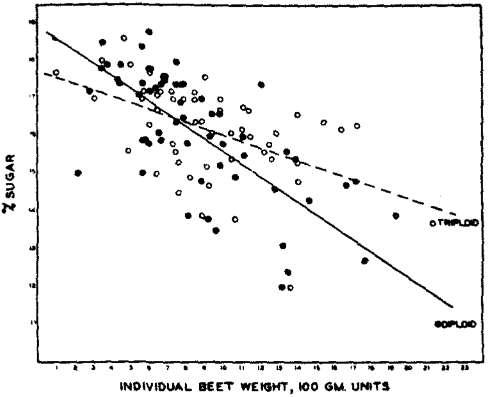

FIG. 1. *Relation between percentage sugar and root weight in individual diploid and triploid beets.*

Yield of Sugar and Root Weight

The most valuable single agronomic characteristic of sugar beets is yield of sugar. Consequently its relation to weight of root is of considerable importance. The previous section, dealing with the regression of percentage of sugar on root weight, showed that the regressions of diploids and triploids had different slopes, though it is possible that the relation is not truly linear throughout the complete range. However, the extent of scatter and low correlation of the two characters limited the interpretation to a linear expression. The determination of the regression of yield of sugar on root weight overcame this difficulty, since these two characters were closely correlated, the r values being $+0.97$ for diploids and $+0.98$ for triploids. The shape of the respective diploid and triploid curves (Fig. 2) was determined by the percentage of sugar, since root weight was a function in both characters under consideration. The diploid and triploid regression lines differed strikingly in degree of curvature beyond the midpoint, at which the diploid curve began to fall away rapidly from the more nearly linear triploid curve. The significance of this difference exceeded the 1% point (Table II) in spite of the fact that the curves were almost identical for beets smaller than 900 gm. It seems possible that the adverse growth conditions resulting from temporary flooding in the area producing the small beets suppressed growth to an extent that tended to mask the difference in the diploids and triploids within this size range.

The difference in the slope of the diploid and triploid regression lines for the larger beets indicates a fundamental difference which may prove to be

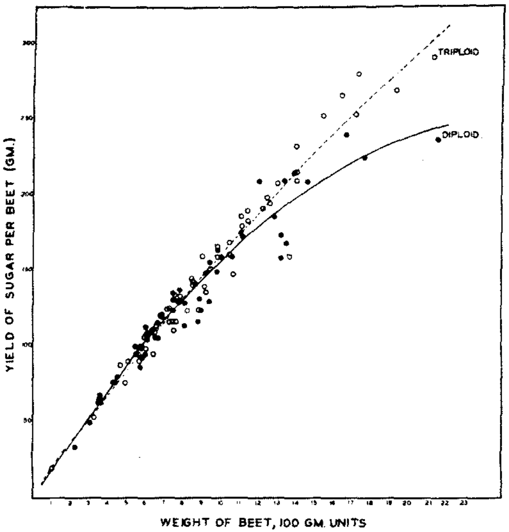

FIG. 2. *Relation between yield of sugar and root weight in individual diploid and triploid beets.*

of considerable practical importance. On the basis of this experiment it should be possible to produce very large triploid beets without any serious reduction in percentage sugar, whereas the economic limit in size might soon be reached in the production of larger diploid beets, where any marked reduction in percentage sugar would counterbalance the advantage of increased root weight.

The Relation of Root Weight and Yield of Sugar to Dry Top Weight

Root weight, as would be expected, was closely correlated with dry top weight, the *r* values for diploids and triploids being +0.86 and +0.89 respectively. The diploid and triploid regression lines for these characters were not significantly different. Similar results were obtained when yield of sugar was correlated with dry top weight, the *r* values for diploids and triploids being +0.83 and +0.87 respectively. The diploid and triploid regression lines for these characters were also very similar.

Comparison of Diploids and Triploids on a Plot Basis

The "t" test (Snedecor 14) was applied to the means of the eight diploid and triploid plots each containing seven plants. The characters studied were root weight, percentage sugar, yield of sugar, and dry top weight. The results are summarized in Table II. Diploid and triploid single row plots were alternated in the field instead of being randomized as required for proper use of the "t" test. In spite of this defect in plot arrangement, the "t" test was considered to be the most useful test since there did not appear to be any consistent positional relation favouring either the diploid or triploid in this closely replicated series. Nevertheless, undue emphasis must not be placed on the significance of the differences derived in this manner.

The plot means are recorded in Table III to show the adjacent plots that were compared. The triploid plot means exceeded their adjacent diploid plot means in root weight, yield of sugar, and dry top weight with one exception in each case, which was accounted for by the presence of one abnormally small triploid beet.

The mean increase of triploids over diploids for root weight was 12.2%, for yield of sugar 14.9% and for dry top weight 17.8%. These increases were significant according to the "t" value shown in Table III, although the

TABLE III

Plot means of diploid and triploid sugar beets

Plot No.	Characters									
	Mean root wt., gm.		Per cent sugar		Yield of sugar		Dry top wt., gm.		Per cent moisture in tops	
	2n	3n	2n	3n	2n	3n	2n	3n	2n	3n
1	1317	1361	14.8	15.0	195	204	153	161	85	85
2	943	964	14.7	15.3	126	145	105	127	83	86
3	580	797	16.2	16.3	92	129	58	75	82	84
4	992	1064	15.5	15.4	147	162	102	125	84	84
5	705	973	16.5	16.1	112	157	54	79	82	83
6	871	991	16.5	16.4	143	158	102	105	84	83
7	585	706	17.6	17.4	102	122	48	78	80	82
8	846	817	16.9	16.9	138	137	104	104	83	83
Mean	854.9	959.0	16.1	16.1	131.9	151.6	90.7	106.8	82.6	83.5
Mean increase of triploids over diploids	104.1		0.0		19.7		16.1		0.9	
Per cent increase	12.2		0.0		14.9		17.8		1.1	
"t" value	2.96*		—		3.80**		4.21**		1.89	

* *Exceeds 5% level (2.36) of significance.*
** *Exceeds 1% level (3.50) of significance.*

percentage increase would likely be very different under more favourable and uniform conditions of growth. The transplanting of well developed seedlings and the unusually long period from germination to harvest may also have affected differentially the diploids and triploids. Consequently additional tests must be made under more normal conditions before the relative qualities of the triploids and diploids could be accurately predicted.

Although the triploids had larger roots on the average than the diploids, the mean percentage of sugar was the same for both. It can be concluded from this that the triploids on the average must have contained a higher percentage of sugar for a given size, as was illustrated by the regression curves.

The diploids and triploids did not differ significantly in regard to percentage moisture in the tops.

Leaf and Stomatal Dimensions

The length and width of the lamina of five of the largest leaves of each plant were taken, and the plot means of these dimensions together with the length-width ratio and area index (length × width) are recorded in Table IV. The triploids exceeded the diploids in length of leaf (lamina) by 15.6%, in width of leaf by 16.8% and in area index by 34.4%. The ratio of length to width was very similar for diploids and triploids, which is in contrast to the observation by Rasmusson and Levan (10) that the triploids have shorter and broader laminae than the diploids.

TABLE IV

PLOT MEANS FOR LEAF DIMENSIONS (INCHES)

Plot No.	Leaf length (in.)		Leaf width (in.)		Ratio L/W		Area index ($L \times W$)	
	Diploid	Triploid	Diploid	Triploid	Diploid	Triploid	Diploid	Triploid
1	8.6	10.8	5.9	6.6	1.54	1.64	50.97	71.11
2	9.1	10.2	5.4	6.3	1.69	1.63	49.50	63.66
3	7.1	9.0	4.8	6.0	1.48	1.50	33.68	54.24
4	9.7	10.4	5.6	6.6	1.72	1.58	54.82	68.73
5	7.9	9.4	4.9	5.9	1.62	1.58	38.83	55.50
6	9.3	10.5	5.8	6.2	1.60	1.68	53.58	65.33
7	7.1	8.6	4.3	5.7	1.64	1.50	30.54	49.34
8	8.3	8.7	5.2	5.6	1.59	1.56	42.87	48.98
	8.39	9.70	5.24	6.12	1.601	1.584	44.349	59.612
Per cent increase		15.6		16.8	1.1			34.4

The plot means for stomatal dimensions are given in Table V. The triploid stomata exceed the diploid in length of the guard cells by 34.6% and in width by 9.3%. The ratio of length to width in the diploids is 1.05 and in the triploids is 1.20, indicating quite a marked difference in shape. However, stomatal shape would vary so greatly with cell turgor that this difference may

TABLE V

PLOT MEANS FOR STOMATAL DIMENSIONS

(1 unit = 1.3 μ)

Plot No.	Length		Width		Area index L × W	
	Diploid	Triploid	Diploid	Triploid	Diploid	Triploid
1	23.6	28.5	20.0	22.3	472.0	635.5
2	21.1	26.9	20.0	21.9	422.0	589.1
3	20.6	26.3	19.0	22.9	391.4	579.4
4	19.4	25.7	18.1	20.9	351.1	537.1
5	22.0	25.7	18.6	22.5	409.2	578.2
6	21.0	27.0	18.3	22.0	384.3	594.0
7	19.7	24.9	18.1	21.5	356.6	535.3
8	20.6	24.4	19.7	20.6	405.8	502.6
Mean	21.00	26.17	19.97	21.82	399.0	568.9
Per cent increase		34.6		9.3		42.6

be of little significance. The stomatal area index of the triploids exceeds the diploids by 42.6%, which parallels the increase of 34.4% in area index of the leaves. The biological significance of this relation cannot be determined at present on the limited information available, but it suggests that the number of cell divisions is similar in diploids and triploids, and that increase in organ size tends to be in direct proportion to increase in cell size.

Discussion

The recent development of artificial methods of inducing chromosome doubling has opened up new possibilities in plant improvement. Chromosome doubling frequently results in increased size of all the plant organs and in addition frequently affects the chemical constitution of plants. Sullivan and Myers (15) found that tetraploid *Lolium perenne* plants contained more reducing sugar and sucrose than the diploid material. Sansome and Zilva (12) found that tetraploid strains of tomato exceeded diploid strains in the vitamin C content. Randolf and Hand (9) report that tetraploid strains of yellow corn were higher in carotinoids than the diploids. Kostoff and Axamitnaja (5) found that tetraploid tomatoes contained a higher proportion of nitrogen and protein than the diploids.

In certain induced autotetraploids the advantage is size, and chemical constitution is frequently nullified by a reduction of seed yield especially in those crops where seed is the merchantable product. However, in the sugar beet the cost of seed is so small in proportion to the cost of production of the roots that a considerable increase in seed cost could be readily borne by any increase in value of the crop through improvement in root yield or quality.

The relative value of tetraploid and diploid sugar beets has not yet been established, but the present investigation indicates that triploids may be

superior to diploids in respect to yield of sugar, weight of root, and particularly *in the maintenance of percentage sugar with increased size of root.* If further tests substantiate these findings, then the development of methods of producing triploid seed on a commercial scale will require considerable attention. Triploids obviously will not breed true, consequently it will be necessary to hybridize tetraploid and diploid strains to provide the triploid seed.

The production of a very high proportion of triploid seeds from open fertilization of the tetraploid flowers in this experiment indicates the ease with which cross fertilization of tetraploids and diploids may be accomplished. This could likely be repeated in large scale plantings by interspacing tetraploids and diploid stecklings in such a manner that most of the seed produced on the tetraploid was the result of pollination by pollen from diploid plants. There is also the possibility that pollen from diploids is more viable on tetraploid stigmas than pollen from tetraploid plants. Such a condition would greatly facilitate the production of triploid seed. A further advantage in obtaining a high degree of cross fertilization would undoubtedly result from heterosis, and parental tetraploid and diploid strains could be selected with the object of obtaining maximum hybrid vigour. The resulting triploid progeny should combine the advantages inherent in triploidy and heterosis.

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EFFECTS OF DUSTS CONTAINING INDOLYLBUTYRIC ACID AND OESTRONE ON THE ROOTING OF DORMANT *LONICERA TARTARICA* CUTTINGS[1]

By N. H. Grace[2]

Abstract

Indolylbutyric acid and oestrone, separately and in combination in a series of talc dusts, were applied to dormant cuttings of *Lonicera tartarica*. Indolylbutyric acid treatment, averaged over all oestrone treatments, increased the number of cuttings rooted, the number and length of roots per rooted cutting, the mean root length, and the green weight of leaf produced by the dormant cuttings. The average effect of oestrone on these responses was depressive throughout. Oestrone tended to offset the beneficial effects of the plant growth stimulating chemical, excepting the combination of 100 p.p.m. of each which increased root length. Oestrone alone did not reduce either the number or lengths of root per rooted cutting.

The results indicated that the number and length of roots and the green weight of leaf produced are more sensitive responses for demonstrating differences due to indolylbutyric acid concentration in talc than is the percentage of cuttings rooted.

An earlier communication has described some effects obtained with dormant cuttings on treatment with indolylbutyric acid and oestrone (2). While the dust treatments with oestrone had no significant effects on the various responses, colloidal solutions of this chemical, both alone and in combination with indolylbutyric acid, affected the weight of green leaf produced by the dormant cuttings. In view of the effects on top growth and the indication of some interaction between these plant and animal hormones, it was considered of interest to carry out an experiment in which both these chemicals were applied, separately and in conjunction with each other, by means of a dust carrier. The present communication describes the results of such an experiment.

Experimental

The effects of the two chemicals, indolylbutyric acid and oestrone, were investigated by an experiment of factorial design. The experiment involved a series of 16 dust mixtures comprising indolylbutyric acid at four concentrations, namely 0, 100, 500, and 1000 p.p.m. (parts of chemical per million parts of talc by weight) in combination with oestrone at the same four concentrations. The individual concentrations and the various mixtures were readily made from a 2000 p.p.m. preparation of each chemical by admixture and addition of ball-milled talc. The 2000 p.p.m. dusts had been prepared by the grinding mix method, a procedure that ensured thorough distribution of the chemical in the carrier.

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Dormant cuttings‡ of *Lonicera tartarica* L. were received on November 2, 1939, and treated in groups of 10 cuttings with the 16 different dust preparations. Prior to dusting the cuttings were sprayed with water (3). There were four replicates of the 16 treatments with complete randomization of order of planting within each replicate; the entire experiment involved the use of 640 cuttings. The dusted cuttings were planted immediately in brown sand in a propagation bed provided with electrical bottom heat cables. The room temperature approximated 65° F. and that of the sand about 70° F. The cuttings were removed for observation on December 18, or 46 days after planting.

A record was made of the number of cuttings rooted in each group of 10, the number and length of the primary roots, and the weight of green leaf per group of cuttings. The mean root length was calculated from the number and total lengths of roots. All the data obtained were subjected to the analysis of variance procedure. The numbers of cuttings rooted were subjected to the inverse sine transformation for statistical analysis (1).

Results

The analyses of variance of the responses are given in Table I. In each case the average effect of indolylbutyric acid treatment attained the 0.1% level of statistical significance. On the average, oestrone treatments were

TABLE I

ANALYSIS OF VARIANCE OF RESPONSE OF DORMANT *Lonicera tartarica* CUTTINGS TREATED WITH DUSTS CONTAINING INDOLYLBUTYRIC ACID AND OESTRONE

Source of variance	D.f.	Mean square				
		Number of cuttings rooted	Number of roots per rooted cutting	Length of root per rooted cutting	Mean root length	Weight of green leaf produced per group of 10 cuttings
Between replicates	3	206.1	7.55**	17,443*	1622.5***	0.3006
Treatments with						
Indolylbutyric acid	3	1376.6***	14.97***	185,422***	3120.8***	5.0989***
Oestrone	3	1244.6***	24.06***	81,637***	407.5†	4.4152**
Interaction						
Indolylbutyric acid × oestrone	9	202.6	7.76***	18,827**	157.1	0.9718
Error	45	111.1	1.71	4,558	157.2	0.6130

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*
† *Partition of the sum of squares indicated that treatment with 1000 p.p.m. oestrone differed from the mean of the other three levels taken together.*

‡ The prepared cuttings were supplied by the Federal District Commission, Ottawa, through the kindness of Mr. E. I. Wood.

also highly significant with measurements on the mean root length an exception. Partition of the sum of squares for the three degrees of freedom for the effects of oestrone treatment on the mean root length indicated that the 1000 p.p.m. oestrone treatment differed from the average value for the other three levels taken together; the significance attained was the 5% level. Significant interaction between chemicals was shown by the observations on numbers and lengths of root per rooted cutting; there was a suggestion of an interaction shown by counts on the number of cuttings rooted but the mean square fell short of the 5% level of significance, a condition not changed by partition of the sum of squares for the interaction.

TABLE II

AVERAGE RESPONSES OF DORMANT *Lonicera tartarica* CUTTINGS TREATED WITH DUSTS CONTAINING INDOLYLBUTYRIC ACID AND OESTRONE

Data are means for 16 groups of 10 cuttings

	Indolylbutyric concentrations, p.p.m. (average over all oestrone concentrations)				Oestrone concentrations, p.p.m. (average over all indolylbutyric concentrations)				Necessary difference, 5% level
	0	100	500	1000	0	100	500	1000	
Number of cuttings rooted									
Transformed data	43.5	60.4	64.9	59.0	69.3	56.1	53.8	48.6	7.5
Per cent	47.5	70.6	78.1	70.0	80.6	66.9	63.1	55.6	
Number of roots per cutting rooted	3.9	4.7	6.1	5.8	6.8	5.1	4.4	4.1	0.93
Length of root per rooted cutting, mm.	67	138	285	279	284	211	154	121	48
Mean root length, mm.	21.5	28.8	47.8	49.8	37.6	41.6	38.9	29.8†	8.9
Weight of green leaves per group of 10 cuttings, gm.	1.78	2.78	3.08	2.34	3.01	2.84	2.09	2.02	0.55

† *See footnote, Table 1. The 1000 p.p.m. concentration of oestrone gave a mean root length of 29.8 mm., the mean of the other oestrone concentrations, 39.4 mm.; both values averaged over all indolylbutyric acid treatments and the only significant effect of oestrone treatment on the mean root length.*

In Table II are given data for the average responses of indolylbutyric acid and oestrone treatments, the result for each chemical being averaged over the four concentrations of the other. All indolylbutyric acid treatments increased the number of rooted cuttings and it is of interest to point out that there was no significant difference in rooting over the range from 100 to 1000 p.p.m., though an optimum was suggested at the 500 p.p.m. level. Oestrone treatment reduced rooting, the 100 and 500 p.p.m. concentrations did not differ between themselves, but the 1000 p.p.m. was below the 100 p.p.m. concentration, suggesting increase in injury to rooting with ascending concentration. Indolylbutyric acid treatment at the 100 p.p.m. concentration suggested an increase in mean root length; the increase became highly signi-

ficant at the two higher concentrations, which failed to differ between themselves. The 1000 p.p.m. oestrone treatment gave a mean root length of 29.8 mm., a value significantly below 39.4 mm., the mean value for the 0, 100, and 500 p.p.m. concentrations taken together. The weight of green leaves per group of 10 cuttings was greater for all treatments with indolylbutyric acid than for the controls; the 500 p.p.m. concentration was followed by the greatest weight of new growth; a significant reduction in the leaf weight over that at the 500 p.p.m. concentration followed the 1000 p.p.m. concentration. While the decrease in leaf weight on treatment with 100 p.p.m. oestrone was not significant, the decrease was marked at the two higher levels, which failed to differ between themselves.

TABLE III

NUMBER OF ROOTS PER ROOTED DORMANT *Lonicera tartarica* CUTTING ON TREATMENT WITH DUSTS CONTAINING INDOLYLBUTYRIC ACID AND OESTRONE

Data are means from 4 groups of 10 cuttings

Oestrone treatments, p.p.m.	*Indolylbutyric acid treatments, p.p.m.*			
	0	100	500	1000
0	3.9	4.9	9.9	8.7
100	4.2	5.3	5.0	6.1
500	4.3	4.5	5.0	3.7
1000	3.4	4.3	4.3	4.6

Necessary difference, 5% level, 1.86 roots.

Data are given in Table III for the interaction effects of indolylbutyric acid and oestrone on the number of roots per rooted cutting. The interaction between chemicals is such that in the absence of oestrone, both the 500 and 1000 p.p.m. treatments of indolylbutyric acid resulted in a number of roots significantly greater than those of the controls. When the 100 p.p.m. oestrone treatment was used only the 1000 p.p.m. concentration of indolylbutyric acid showed a significant increase in the number of roots over the controls, while the 500 and 1000 p.p.m. concentrations of oestrone completely offset any beneficial effect of indolylbutyric acid. Since none of the oestrone treatments in the absence of indolylbutyric acid had a significant effect, the apparent reduction in the number of roots on treatment with this chemical, as shown by the average values in Table II, must be attributed to the interaction with the plant hormone concentrations.

Data are given in Table IV for the interaction effects of indolylbutyric acid and oestrone on the length of roots per rooted cutting. In the absence of oestrone, indolylbutyric acid increased root length markedly, the 500 p.p.m. concentration was followed by a greater length of root than either the 100 or 1000 p.p.m. treatments and the results suggest that the highest level is past

TABLE IV

LENGTH OF ROOTS (MM.) PER ROOTED DORMANT *Lonicera tartarica* CUTTING ON TREATMENT WITH DUSTS CONTAINING INDOLYLBUTYRIC ACID AND OESTRONE

Data are means from 4 groups of 10 cuttings

Oestrone treatments, p.p.m.	Indolylbutyric acid treatments, p.p.m.			
	0	100	500	1000
0	72	162	469	432
100	89	221	247	286
500	59	125	254	179
1000	49	43	172	219

Necessary difference, 5% level, 24 mm.

the optimum. In the absence of indolylbutyric acid the 100 p.p.m. concentration of oestrone suggested an increase in root length; while neither the 500 nor the 1000 p.p.m. concentrations were significantly below the control, both were below the 100 p.p.m. concentration in respect to root lengths. In conjunction with 100 p.p.m. indolylbutyric acid the same concentration of oestrone increased root length, but the two higher concentrations reduced length markedly. The greatest reduction occurred at the 1000 p.p.m. concentration. In conjunction with 500 p.p.m. indolylbutyric acid, oestrone reduced length of roots, and the greatest reduction followed use of the highest concentration; in conjunction with 1000 p.p.m. indolylbutyric acid a somewhat similar condition was found in that all eostrone treatments depressed root length. However, while the 100 and 1000 p.p.m. oestrone treatments are significantly less damaging in conjunction with 1000 p.p.m. indolylbutyric acid than with the 500 p.p.m. concentration, the reverse is true of these combinations with the 500 p.p.m. oestrone concentration. In consequence, the depressive effects for oestrone treatments, as shown by the average values in Table II, may be attributed largely to its combination with the plant hormone indolylbutyric acid; the main effect was to decrease the root lengths that followed treatment with plant hormone; in one instance, at 100 p.p.m. of each in conjunction, an increase in root length was obtained.

It was pointed out that the interaction between indolylbutyric acid and oestrone on the number of cuttings rooted approached statistical significance. Examination of the data indicated that while oestrone treatment, averaged over all indolylbutyric acid treatments, reduced rooting as shown in Table II, this was to be attributed in part to its combination with the several indolylbutyric acid concentrations. In the absence of indolylbutyric acid, the three oestrone concentrations gave lower rooting than talc only, but none of them to a significant extent though depression of rooting increased with ascending oestrone concentration. Depression of rooting occurred to somewhat the same extent when oestrone was combined with the 100 and 1000 p.p.m.

concentrations of indolylbutyric acid, to a slightly lesser extent in combination with the 500 p.p.m. concentration. The cumulation of these effects accounts for the over-all depression of rooting shown by the oestrone treatments.

Conclusion

The results of this experiment are in general agreement with those described in an earlier communication in which *Lonicera tartarica* cuttings were treated with talc dusts containing three levels of both indolylbutyric acid and oestrone, each separately (2). In this former experiment cuttings were taken later in the season and after severe frosts, facts which, combined with absence of provision for study of the interaction of the chemicals, make close comparison of the results of the two experiments impossible. The present experiment has shown that the plant hormone has stimulated the rooting, number, and length of root per rooted cutting, the mean root length, and the green weight of leaf produced by the dormant cuttings. The marked stimulation of top growth by indolylbutyric acid treatments is particularly interesting. It may be pointed out that the numbers and lengths of roots and the weight of green leaf produced are relatively more sensitive methods of determining the effects of different hormone concentrations in dust than mere counts of the number of cuttings rooted. While there was no significant difference in percentage rooting at 100, 500, and 1000 p.p.m. indolylbutyric acid, the other responses showed the presence of marked effects attributable to concentration.

Oestrone treatments had a depressing effect throughout, though to a considerable extent the depressive tendency was related to the interaction with indolylbutyric acid. The results suggest that, in the concentration range investigated, oestrone tended to offset the beneficial effects of the plant growth stimulating chemical. However, the combination of 100 p.p.m. of each chemical had a beneficial effect on root length per rooted cutting, and it would appear that further experiments on oestrone and plant hormones in talc should deal with oestrone concentrations below 100 p.p.m.

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THE EFFECT OF CROP DEBRIS ON THE PATHOGENICITY OF CEREAL ROOT-ROTTING FUNGI[1]

By L. E. Tyner[2]

Abstract

The effect of wheat, oat, and barley straw, composted with soil, on the development of disease on the basal parts of wheat seedlings was studied in a series of greenhouse experiments during three seasons. The pathogens used were *Ophiobolus graminis* Sacc., *Helminthosporium sativum* P. K. and B., and *Fusarium culmorum* (W. G. Sm.) Sacc. The wheat-straw composts were distinctly more favourable to the development of disease than the composts of either oat or barley straw. The least injury occurred on seedlings grown in composts of oat straw. It is suggested that the micro-organisms associated with the decomposition of oat straw bring about some degree of biological control of the plant pathogens also present.

Although the amount of straw applied sometimes influenced severity of disease, the effects were not consistent from planting to planting. Apparently the actual carbon to nitrogen ratio had less effect upon disease development than did the chemical nature of the straw.

The kind and amount of straw in the composts also influenced seedling vigour. This vigour was, in general, inversely proportional to the degree of infection.

The introduction of a short fallow period between plantings decreased infection somewhat and increased vigour.

The pathogenicity of the artificial inoculum added at the first planting was practically vitiated before the second planting. Subsequently, the infection ratings tended to increase and were about the same as those in the uninoculated series.

Introduction

It is common observation that the development and severity of the root-rot diseases of wheat caused by the soil-inhabiting pathogens, *Ophiobolus graminis* Sacc., *Helminthosporium sativum* P. K. and B., and *Fusarium culmorum* (W. G. Sm.) Sacc., are favoured much more by certain crop sequences than by others. Recent experimental evidence has indicated that complex biological antagonisms and other obscure phenomena are, in part, responsible for the control indicated or for the lack of it, as the case may be.

Among the many factors that may favour or retard the degree of control resulting from a given crop, the kind and amount of crop residue returned to the soil should play an important role. On the other hand, the result in field soil, where the residue is probably subjected to a more complex biological environment, may be quite different than it is in artificial culture. Moreover, it is conceivable that the crop residue is not more important in determining the growth and virulence of the pathogens than the particular ecological conditions associated with a given crop or a bare summerfallow. Experimental evidence of the chain of events that occur in either case is almost wholly lacking.

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The object of the present study was to examine the differential effect in natural soil in the greenhouse of wheat straw, oat straw, and barley straw on the development of the root rots of wheat caused by *Ophiobolus graminis*, *H. sativum*, and *F. culmorum*.

Literature

The organic matter of the soil is a factor of importance in crop production. Schafer *et al.* (20) concluded that a carbon to nitrogen ratio in excess of 12 : 1 provided unfavourable conditions for crops. Greaves and Hirst (10) showed that it was possible to add all crop debris to the soil if the rotation chosen was effective in maintaining the nitrogen of the soil. Gray and McMaster (8) found that the activity of micro-organisms was dependent on the organic-matter relations in podsol soils. Waksman and Starkey (27), and Anderson (1) pointed out that the growing plant was quite unable to compete with micro-organisms in utilizing nitrogen when the cellulose content of the soil was high. When wheat straw was added to the soil, Murray (14) and Vandecaveye and Allen (22) found marked and immediate increases of bacteria, and, within a week, similar increases of fungi. The numbers of both groups fell off rapidly after 60 days at 20° C.

Any estimation of the rate and manner of decomposition must be made under conditions as nearly natural as possible. No significance may be attached to results obtained with one organism, for, as shown by Norman (15) and Waksman *et al.* (25), there is a far different effect from a mixed flora than is observed when only one organism is present. These mixed flora are of particular interest to the plant pathologist who is studying soil-inhabiting pathogens, because in the last analysis the virulence of a given pathogen or any increase or decrease therein must depend upon the associated microflora. Thus, Sanford (18) suggested that the control of potato scab in certain soils by incorporating green rye was attributable to the antibiotic effect, on *Actinomyces scabies*, of soil micro-organisms whose development was stimulated by the presence of green manure. The frequently observed (3, 4, 6, 7, 9, 16, 17), rapid diminution in the pathogenicity of *O. graminis*, *H. sativum*, or *F. culmorum*, added to soil in the presence of excess organic matter, is likewise probably attributable to the antagonism and competition of other soil-inhabiting micro-organisms stimulated by the organic matter added.

Materials and Methods

The experiments reported here were started in the greenhouse in the fall of 1935. Straw of wheat, oats, and barley was secured after harvest from contiguous plantings and reduced to the fineness of chop. The ground straw was then incorporated with a 3 : 1 soil-sand mixture in the proportions of 1.6, 3.1, and 4.7% by weight, and placed in six-inch, unglazed flower pots. The pots containing the mixtures indicated, and also a control lot filled with soil only, were placed on greenhouse benches for 17 days, during which they were kept moist. At the end of this period 20 surface-disinfected seeds of

Marquis wheat, previously graded for size, were sown. All treatments were replicated four times. At the beginning of the experiments, soil-grown inoculum of *O. graminis*, *H. sativum*, or *F. culmorum* was added with the seed in ten-gram quantities. In preparing the soil inoculum, all but a minimum of organic matter was excluded. In the case of *O. graminis*, air-dry soil was mixed with 5% corn meal by weight, 250 cc. of potato water from 125 gm. potatoes per litre of water, and 20 gm. of dextrose per kg. of dry soil. In preparing the inoculum of *H. sativum* and *F. culmorum* only corn meal was added to the soil. These media were steam sterilized, then inoculated and incubated for three weeks at room temperature.

The position of the pots in each replicate was changed weekly to eliminate local differences in temperature. A heavy wire screen was placed beneath the pots to assist aeration and hinder contamination from below by capillary suction. When the pots were being watered, care was taken to prevent run off. At the end of 40 days the severity of disease on each seedling was estimated numerically in a range of 0 to 10. Parallel series of oat and barley seedlings were planted, but for the sake of brevity the results are not reported in this paper.

The 1935–1936 Experiment

First Three Plantings

During the first three plantings of this experiment additional inoculum was not applied for the second and third crop. The pots were simply replanted immediately after each crop of wheat seedlings was removed.

In the first planting there was slightly more disease in the *H. sativum* series than in the corresponding *F. culmorum* series. In the *O. graminis* series it was about half as severe as in the *H. sativum* series, but approximately twice as great as that in the uninoculated soil-straw controls. A relatively low percentage of plants survived in the *H. sativum* and *F. culmorum* series, which indicated that both pathogens were quite active under the conditions. The survival value in the *O. graminis* series was slightly less than in the uninoculated control. Apparently there was no consistent effect of the kind or amount of straw on the disease ratings in any of the series (Fig. 1 C).

In the second planting, disease in the *H. sativum* and *F. culmorum* series was only about one-third as severe as in the first planting, and in the *O. graminis* series about half as great. The amount in the uninoculated controls was approximately the same as in the first planting and not significantly different from that in the inoculated series. The number of surviving plants was normal in all series, which indicates that all three pathogens were less active than in the first planting. There was a tendency for more disease in the wheat-straw compost than in the other composts, and also possibly a slight tendency for more disease in the highest concentration of straw (Fig. 1 C).

In the third planting the disease was approximately the same as in the preceding planting in all series, with the exception of the *H. sativum* series, where it was somewhat greater. With regard to the *O. graminis* series, it is known

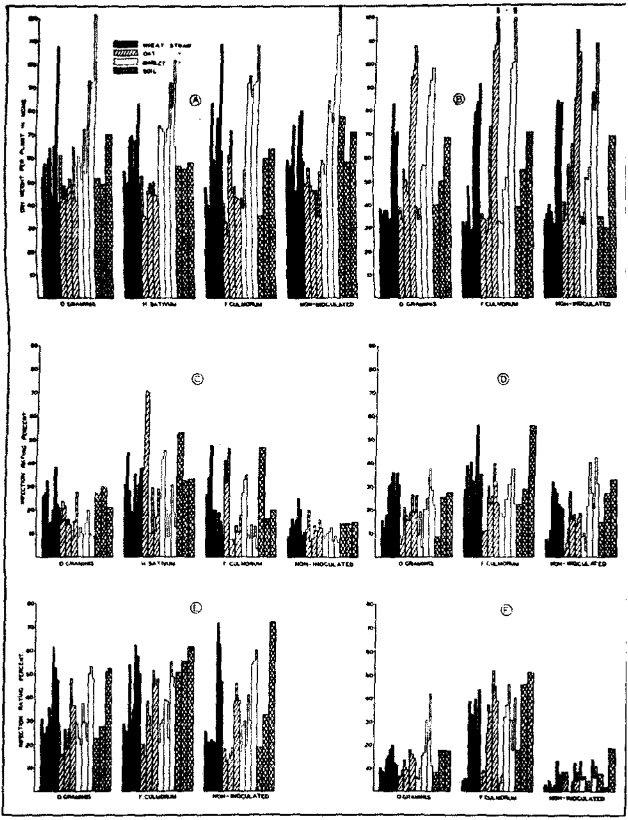

FIG. 1. *Effect of wheat, oat, and barley straw in three different amounts (1.6, 3.1, and 4.7%) in inoculated soil-straw composts, on the vigour and disease of wheat seedlings. These effects are illustrated from three plantings of three separate experiments, as follows:* A *and* C, *dry weight and disease, respectively, in plantings 1, 2, and 3, 1935 experiment.* B *and* D, *dry weight and disease, respectively, in plantings 1, 3, and 5, 1936 experiment.* E *and* F, *disease in unsterilized and sterilized soil series, respectively, in plantings 4, 5, and 6, 1936 experiment. Each large column represents a planting in the order named, and the three subdivisions of each column the variations for each straw concentration in order mentioned.*

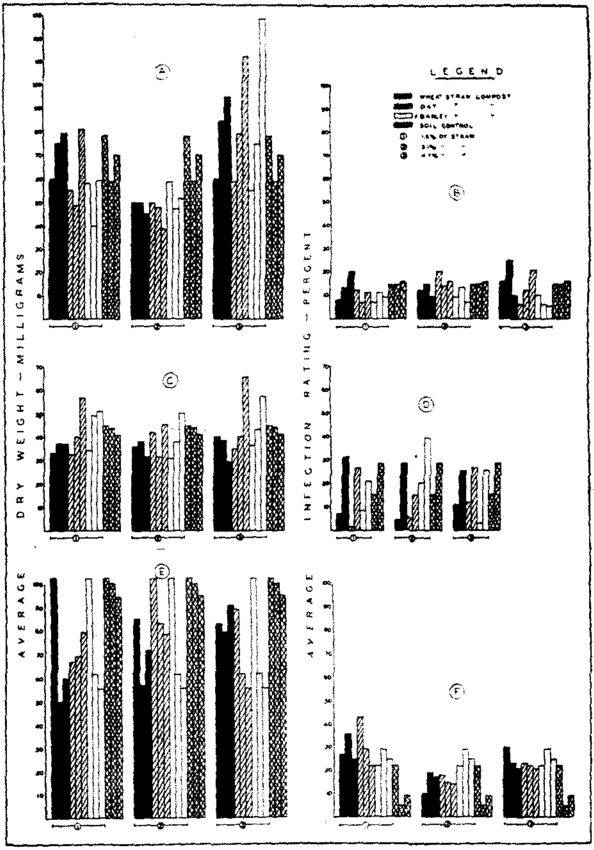

FIG. 2. *Effect of wheat, oat, and barley straw in three different amounts (1.6, 3.1, 4.7%) in uninoculated soil-straw composts, on vigour and disease of wheat seedlings. These effects are illustrated for the first, second, and third plantings of three separate experiments, namely, 1935,* A *and* B; *1936,* C *and* D; *and 1938,* E *and* F. *Each column represents a planting.*

that both the virulence and the persistence of the inoculum of this pathogen are quickly vitiated when added to either sterilized or natural soil (2, 16, 19). Therefore, the disease observed in this series was probably caused either by *H. sativum* or *Fusarium* spp., or both. In this connection it is significant that, with the exception of the wheat-straw compost, there was approximately the same amount of disease in the *O. graminis* series as in the uninoculated control. Also, there was again a tendency for more disease in the wheat-straw compost than in the corresponding oat and barley composts. The higher concentration of wheat straw favoured more disease in the *H. sativum* series, but less in the other series. With oat straw, there was an increase in disease at the higher concentrations in all series, but in the barley compost there was a decrease (Figs. 1 C, 2 B). The number of surviving plants was approximately normal in the barley compost in all series, but quite low in the oat compost, and in the two highest concentrations of wheat straw in all inoculated series. The reasons for this are at present obscure.

With regard to plant vigour in the first three plantings, there was, with a few exceptions, a close relation between the amount of disease and the dry weight of plants. An increase in infection rating was correlated with a decrease in dry weight of the plants. The relative fertilizing values of the three kinds of straw composts are indicated by the dry weight of the plants in the control series. For example, in the first planting growth was inhibited in all composts to about the same extent. However, in the second and third crops the growth was increased on the barley-straw compost, but decidedly inhibited in the oat-straw compost. In the former case, growth increased with the concentration of straw, but in the latter all concentrations were equally inhibitory. Growth on wheat straw in the second planting was stimulated in the lowest concentration and inhibited in the higher ones. In the third crop the highest concentration inhibited growth, whereas the two lower ones stimulated it (Figs. 1 A, 2 A).

Fourth Planting

When the third planting was harvested, the experiment was modified in certain particulars. The pots in the *F. culmorum* series were treated with a 1 : 500 solution of mercuric chloride at the rate of 200 cc. per pot, while the pots of the uninoculated control series were steam sterilized. The two series originally inoculated with *O. graminis* and *H. sativum* were continued. In addition, all pots were allowed to remain fallow for two weeks. Further, the nitrate nitrogen was determined in the various 4.7% straw composts of the *O. graminis* series. For this the Spurway (21) spot-plate method was used. Tests were made from samples taken as soon as the third planting was harvested and again just before planting at the end of the fallow period. The nitrate nitrogen is listed below in parts per million of soil for each compost and the soil control, at the beginning and end of the test, respectively:

Wheat straw	Trace	9.25
Oat straw	0	Trace
Barley straw	Trace	16.6
Soil control	0	5.43

The foregoing data are of special interest in comparing the vigour of the seedlings of wheat on composts of wheat, oat, and barley straw in the third and fourth plantings. The test indicates that the available nitrate nitrogen was deficient at the end of the third planting.

The disease rating on wheat in the *O. graminis*, *H. sativum*, and *F. culmorum* series was essentially the same as in the third planting. Apparently neither the short fallow period to which all the series were subjected nor the treatment with mercuric chloride solution given the *F. culmorum* series, increased or decreased the disease. In general, considerably more disease continued to develop on the wheat-straw composts than on those of oat or barley straw. In view of the general increase observed in nitrate nitrogen during the fallow period, it would appear that this factor did not affect the level of disease. The plants in the steam-sterilized series were practically clean. Further, more plants survived than in the third planting. Possibly this was related to the general increase in fertility during the fallow period.

Plant vigour was greatly increased in all composts and soils in both the unsterilized and steam sterilized series. In the unsterilized series, the plants from the barley-straw composts were more vigorous than those from the corresponding wheat-straw composts, and those from the oat-straw composts were very much less vigorous, or, in general, about the same as those from the soil control. All steam-sterilized straw composts, and also the sterilized soil control, produced much more vigorous plants than the corresponding unsterilized units, and it is particularly significant that the yield on the oat-straw composts was somewhat greater than it was on either of the other two composts.

The fact that steam-sterilized oat-straw compost indicated greater fertility than those of wheat or barley straw under identical treatment suggests that the potential plant nutrients of the former had been from the beginning as great as in the latter composts, but that obscure biological processes associated with the decomposition and liberation of plant nutrients had been materially different in each case. The data on plant vigour and disease are given in Table I.

Fifth and Sixth Plantings

The main object in the fifth planting was to study the effect of fallow on disease and plant vigour. At the conclusion of the fourth planting the composts containing the three different percentages of each kind of straw were mixed to give for each an average of approximately 3% of straw, as based on the original composts. The procedure was followed for the two series originally inoculated with *O. graminis* and *H. sativum*, and the uninoculated series, which had been steam sterilized in the fourth planting. The *F. culmorum*

TABLE I

EFFECT OF VARIOUS STRAW-SOIL COMPOSTS ON VIRULENCE OF THREE ROOT-ROTTING FUNGI AND ON PLANT VIGOUR IN FOURTH CROP OF WHEAT SEEDLINGS FROM ORIGINAL INOCULATION OF COMPOSTS

Compost	Per cent	Average infection, %	Average height, cm.	Average dry weight, mg.
O. graminis				
Wheat	1.6	29	31	101
	3.1	23	38	168
	4.7	15	39	131
Oat	1.6	4	25	65
	3.1	13	31	80
	4.7	2	26	71
Barley	1.6	10	38	131
	3.1	4	36	128
	4.7	9	40	143
(Soil only)		16	36	100
H. sativum				
Wheat	1.6	37	36	112
	3.1	23	39	126
	4.7	19	32	90
Oat	1.6	13	34	110
	3.1	13	30	92
	4.7	9	26	67
Barley	1.6	30	32	98
	3.1	12	36	142
	4.7	8	40	146
(Soil only)		37	33	90
*F. culmorum**				
Wheat	1.6	22	38	116
	3.1	10	40	130
	4.7	17	37	104
Oat	1.6	4	38	107
	3.1	3	34	82
	4.7	4	36	103
Barley	1.6	7	39	119
	3.1	5	41	133
	4.7	2	42	141
(Soil only)		25	33	68
Uninoculated Control†				
Wheat	1.6	4	41	171
	3.1	2	38	166
	4.7	0	47	201
Oat	1.6	0	43	203
	3.1	0	46	230
	4.7	0	44	208
Barley	1.6	0	44	190
	3.1	1	43	175
	4.7	1	43	219
(Soil only)		1	41	163

* *Soil drenched with 200 cc. of 1 : 500 mercuric chloride solution per pot.*
† *Steam sterilized.*

series was discontinued. Thus, from each of the three kinds of straw composts in each of the three series indicated, there were prepared 20 pots for planting. Beginning May 26, and each week thereafter for four weeks, four pots of each straw compost from the *O. graminis* and *H. sativum* series, respectively, were inoculated with the pathogens and, with an equal number from the control series, planted to wheat. The remaining pots were thus in a state of fallow for one, two, three, or four weeks, as the case may be. The usual notes on disease and plant vigour, which were taken on the first four of the five plantings at the end of 35 days, are listed in Table II.

TABLE II

EFFECT OF FALLOWING VARIOUS STRAW-SOIL COMPOSTS FOR ONE, TWO, THREE, OR FOUR WEEKS ON PATHOGENICITY OF TWO ROOT-ROTTING FUNGI TO WHEAT SEEDLINGS

Compost	Weekly average per plant											
	Infection, %				Height, cm.				Dry weight, mg.			
	1	2	3	4	1	2	3	4	1	2	3	4
O. graminis												
Wheat	38	29	32	33	26	26	26	28	86	81	86	82
Oat	10	5	8	5	25	25	29	30	52	61	75	72
Barley	27	22	17	19	26	27	30	32	79	88	69	91
(Soil only)	32	25	30	30	23	26	22	23	51	70	50	47
H. sativum												
Wheat	33	31	30	29	27	28	31	30	76	87	73	89
Oat	31	21	17	15	23	25	28	32	56	87	74	110
Barley	34	27	28		28	28	29		81	93	89	
(Soil only)	34	29	25	26	22	23	28	24	46	70	94	44
Uninoculated												
Wheat	12	4	5	5	34	32	35	39	135	128	148	149
Oat	10	1	3	3	24	27	27	29	68	87	73	77
Barley	5	2	2	4	32	35	34	35	149	128	135	134
(Soil only)	13	10	12	16	33	30	29	33	140	99	90	101

Analyses for nitrate nitrogen in each kind of compost and soil in the *O. graminis* series were made before each planting. The phenol-disulphonic-acid colorimetric method was used. The results are listed in Table III. The first analysis revealed only a trace of nitrate nitrogen, but during the next week there was a relatively large increase. This increase was greatest with the wheat straw, but less marked in the barley and oat straw. The nitrate nitrogen in the soil control was much less in all succeeding tests than in the composts. Although the general order of increase mentioned was not maintained consistently in subsequent tests, more nitrates were found in both the

TABLE III

NITRATE NITROGEN* (P.P.M.) IN VARIOUS STRAW-SOIL COMPOSTS AS AFFECTED BY PERIOD OF FALLOW

Compost	Fallow period in days				
	0	7	14	21	28
Wheat	Trace	3.74	4.03	3.0	5.25
Oat	Trace	1.75	5.0	Trace	1.92
Barley	Trace	2.65	5.21	3.9	5.65
(Soil only)	Trace	0.75	1.38	Trace	1.62

* *The phenol-disulphonic acid method was used.*

wheat- and the barley-straw composts than in the oat-straw compost. In the latter case it was only slightly greater than in the soil control.

The disease on all weekly plantings was approximately the same, the only differences being with respect to the different straw composts. The seedlings on the oat-straw compost had consistently less disease than those on the other composts, or those on the inoculated soil control. This reduction appears to be related in some way with the associated microflora in the oat-straw compost. At any rate, it would seem that it is not a result of the low fertility indicated by the nitrate analysis, because disease was considerably more severe on the inoculated soil control which contained even less nitrate nitrogen than the oat-straw compost.

With regard to plant vigour, there was a definite tendency for an increase on all composts in each of the four plantings. There was no marked change in the soil control. Vigour of the seedlings on the oat-straw composts and the soil control series was less than on the wheat and barley composts. The only reason apparent from the data is that where an increase in vigour occurred in the two inoculated series it was related to an increase in nitrates.

Data on the seedlings in the uninoculated series, which had been steam sterilized in the preceding experiment, are of special interest, because they confirm the results obtained from the two inoculated series just discussed. For example, vigour was again decidedly less on the oat-straw composts than on either the wheat-straw or barley-straw composts, or even on the soil control, despite the fact that, in the preceding planting, growth on this oat-straw compost had exceeded that on either of the other two composts.

The effect of fallow was observed again in the sixth planting. Two weeks after harvest of the seedlings, tests were made by the Spurway method of the active nitrate nitrogen, potassium, and phosphorus in the various units of the experiment (Table IV).

In general, the soil controls in both inoculated and uninoculated (steam sterilized at fourth planting) series indicated less nitrogen, phosphorus, and potassium than any of the straw composts. The nitrate nitrogen was highest in the wheat straw composts, but it was approximately as low in the oat compost as in the soil control.

TABLE IV

NITRATE NITROGEN, POTASSIUM, AND PHOSPHORUS* IN VARIOUS STRAW-SOIL COMPOSTS TWO WEEKS AFTER HARVESTING THE SIXTH SUCCESSIVE CROP OF WHEAT SEEDLINGS

Compost	Treatment	Nitrate nitrogen, p.p.m.	Potassium, p.p.m.	Phosphorus, p.p.m.
Soil	*O. graminis*	Trace	5	0.5-1
	H. sativum	2	5	0.5-1
	Uninoculated	2-5	0-5	0.5-1
Wheat	*O. graminis*	0-2	5	0.5-1
	H. sativum	5-10	10	1-2.5
	Uninoculated	10-25	10	1-2.5
Oat	*O. graminis*	0-2	5	0.5-1
	H. sativum	5	10	0.5-1
	Uninoculated	Trace	5-10	1-2.5
Barley	*O. graminis*	2	5-10	1-2.5
	H. sativum	2	10	1-2.5
	Uninoculated	2-5	0-5	1-2.5

* *As indicated by the Spurway practical soil testing method.*

Nitrates were highest in the uninoculated pots containing wheat straw, barley straw, or soil only. Obviously the store of nitrate nitrogen released by sterilization had been maintained, at least in part, in these cases. However, nitrates were lowest in the uninoculated pots containing oat straw. This difference affords a reasonable explanation of the much reduced vigour of plants grown on the oat-straw composts in the fifth and sixth plantings. It is not known why the fertility of the oat-straw composts should be so much reduced, but a possible explanation is that it is related to differences in the microbiological conditions in the presence of the various straws. No noteworthy trends in the occurrence of phosphorus or potassium were apparent for any of the straw composts from these tests.

THE 1936–1937 EXPERIMENT

A new experiment, designed on the same general lines as that begun in the fall of 1935, was started in the fall of 1936. Uniformly mature straw of wheat, oats, and barley from a different locality was secured for making the various composts, and the same percentages of straw, viz., 1.3, 3.1, and 4.7%, were added to soil as in the previous experiment. The 4.7% series of barley straw was omitted from the *H. sativum* and *F. culmorum* series on account of insufficient material. The composts were originally inoculated with subcultures of the same strains of *O. graminis*, *H. sativum*, or *F. culmorum* that were used in the 1935 experiment. The usual data were recorded at the end of 40 days and the pots were replanted immediately.

First, Second, and Third Plantings

The results on wheat seedlings for the first three plantings are illustrated in Figs. 1 B and D, and 2 C and D.

Only a slight infection developed in the first planting. In fact, with the exception of the *F. culmorum* series, it was very little greater than in the uninoculated series. A light infection developed again in the second planting, but in the third one a moderate infection developed in all series, regardless of whether they had been previously inoculated or not. Apparently the disease was not caused by the artificially added pathogens. Decidedly less infection occurred on seedlings grown in the oat-straw composts than on the wheat- and barley-straw composts.

All composts appeared equally favourable to plant vigour in the first planting, but in the second and third plantings the wheat-straw composts were definitely deleterious, and this effect was most pronounced in the 4.7% units. In general, barley straw was more favourable than oat straw, but both were more favourable than the soil control. The height of the plants often varied greatly from one planting to another, according to the seasonal intensity of light, and, therefore, only variations in height in each planting are comparable. For this reason, dry weight indicated relative vigour more accurately than plant height.

Fourth Planting

It will be recalled that in the fourth planting of the 1935 experiment the effect of steam sterilization was studied by making certain adjustments. This time the *H. sativum* series was discarded, but the *O. graminis*, *F. culmorum*, and uninoculated series were continued. Half the pots in each series were steam sterilized, and these, with the corresponding unsterilized units, were reinoculated with *O. graminis* or *F. culmorum* as required. For reinoculation, 10 gm. of soil-grown inoculum of the pathogen was added with the seed at time of planting. The data on infection ratings are given in Fig. 1 E and F.

In the inoculated series of the unsterilized section, the amount of disease was practically the same as in the third planting, and was not significantly greater than in the uninoculated control. Also, plant vigour was relatively the same as in the third planting. The seedlings grew best on the oat-straw composts, and they were least vigorous on the wheat straw. Vigour increased with increasing amounts of oat or barley straw in the composts, but tended to decrease as the proportion of wheat straw increased. The least growth occurred on the soil controls. Obviously all three kinds of straw compost were more fertile than the soil alone.

In the steam sterilized, reinoculated series, however, disease was practically absent. Apparently the amount of inoculum applied was insufficient to be effective under these conditions. Evidence on this point will be found in the next planting. Plant vigour was greatly stimulated by sterilization. The greatest growth of seedlings occurred on the composts containing the least straw, which is opposite to what occurred in the unsterilized series. Wheat straw in the 4.7% proportion was the least stimulatory. Again, as in the unsterilized section growth was greater in most cases on all the straw composts than on soil alone.

Fifth Planting

The purpose of this planting was to obtain further data on the effect of steam sterilization of the composts on disease and plant vigour, and to study the influence of a two-week fallow period.

After taking up the seedlings from the fourth planting all pots were fallowed for two weeks. Half the pots were then again steam sterilized. The inoculated series was reinoculated this time with 20 gm. of the soil inoculum of *O. graminis* or *F. culmorum*. The data on disease and dry weight are illustrated in Fig. 1 B, D, E, and F.

In the unsterilized set, inoculation by either organism caused significant infection only in the wheat-straw composts of both series, and in the soil control only in the *F. culmorum* series. There was less disease on seedlings grown in oat-straw composts than in those of wheat or barley straw, or in the soil control of all three series. There was no definite relation between disease and amount of straw used in the three series, but quite marked differences were apparent in individual cases. There were 16% fewer surviving plants in the *F. culmorum* series than in the *O. graminis* series.

There was a marked increase in vigour over the fourth planting. This, undoubtedly, was caused by the period of fallow. Dry weight was greatest on the composts of oats and barley straw but was reduced by more than 25% when wheat straw was used. All three kinds of compost supported much more growth than the soil only.

In the sterilized units, the artificial inoculation by both *O. graminis* and *F. culmorum* was fairly effective. *F. culmorum* caused over twice as much disease as the former organism, and, in addition, decreased the number of surviving plants by 15% more than *O. graminis*. In both inoculated series, disease was about the same on seedlings grown in oat-straw and wheat-straw composts, and approximately 19% higher on composts of barley straw. The amount of straw had no consistent effect on disease in this test.

The plants were slightly more vigorous than in the fourth planting. This was probably a result of the period of fallow given. Dry weights were about 10% less in the *F. culmorum* series than in either the *O. graminis* or the uninoculated series. In the latter series the most vigorous plants were on the oat-straw compost and the least vigorous ones on barley straw. The differences in growth between the sterilized and unsterilized series were greater on the wheat-straw composts than on the other two composts. Vigour was not consistently affected by varying the amount of straw, but was always greater on the composts than in the soil control.

Sixth Planting

The purpose of this planting was to observe further the effect of sterilization on disease and plant vigour, and to note the effect of continued cropping on the increase of disease on plants in composts never artificially inoculated.

Immediately after taking up the fifth planting, the pots that had been steam sterilized in the two previous plantings were again sterilized, and the

corresponding section that had never been sterilized was continued as before. To the pots of the inoculated series in both sections was again added 20 gm. of inoculum of *O. graminis* or *F. culmorum*, as required. All pots were reseeded to wheat. The data on disease are illustrated in Fig. 1 E and F.

The severity of the disease in the unsterilized section of the *F. culmorum*, *O. graminis*, and uninoculated series, increased over that in the corresponding series of the fifth planting by 41, 80, and 135%, respectively. Also, the infection rating was as high in the artificially inoculated series as in the uninoculated series, which, in view of the almost complete absence of disease in the previous planting, is an interesting, but by no means unusual situation in experiments of this sort. Disease was slightly decreased by the oat-straw composts, as indicated by the average rating of 43% in comparison with 53% and 57% for the barley and wheat-straw composts, respectively.

Plant vigour averaged approximately 45% less than in the fifth planting. Although some of this was due to the increase in disease, most of the difference was probably traceable to the fallow period prior to the fifth planting. The barley-straw composts favoured growth slightly more than either of the others. Also, growth appeared slightly increased at the higher concentrations of straw in the wheat and barley composts. There was no difference in the oat-straw composts.

With regard to the sterilized series, both *O. graminis* and *F. culmorum* were considerably more virulent than in the unsterilized series, and the latter pathogen caused twice as much disease as the former. The oat-straw composts favoured disease more than either the barley or the wheat composts, which is contrary to the results consistently obtained in the unsterilized soil in all previous tests. Although neither the kind nor the amount of straw in the composts indicated any very marked differential effect on plant vigour, the fertilizing value of it was evident in comparison with the soil control.

The 1938–1939 Experiment

The work was repeated to obtain further evidence regarding the effect of wheat-, oat-, and barley-straw composts on *H. sativum*, *F. culmorum*, and *O. graminis*.

The composts were each prepared as in previous experiments. Only two proportions of straw were continued, namely, 1.6 and 4.7%. The pots were inoculated only once, just before seeding in the first planting. Two kinds of soil controls were used, namely, one inoculated at the beginning and carried throughout all plantings, and another freshly prepared and inoculated for each succeeding planting. The usual parallel inoculated and uninoculated portions of each compost and soil control series were maintained.

In general the results of this experiment confirmed those of the first and second experiments. For example, the severity of disease in the inoculated and uninoculated series was not significantly different. The average disease ratings in the inoculated series on the oat-, barley-, and wheat-straw composts

TABLE V

THE EFFECT OF VARIOUS STRAW-SOIL COMPOSTS ON THE VIRULENCE OF THREE ROOT-ROTTING FUNGI AND ON PLANT VIGOUR DURING FIVE SUCCESSIVE CROPS OF WHEAT SEEDLINGS

Compost	Per cent	Av. infection, %					Av. dry weight, mg.				
		1	2	3	4	5	1	2	3	4	5
O. graminis											
Wheat	1.6	24	33	27	35	23	125	76	63	50	44
	4.7	33	23	28	29	27	71	101	82	67	60
Oat	1.6	19	35	26	37	26	100	83	72	53	47
	4.7	17	25	31	28	9	96	101	73	52	46
Barley	1.6	25	22	26	35	16	103	88	62	48	42
	4.7	20	26	19	34	22	103	96	75	60	47
Soil*		38	34	36	52	29	92	70	60	45	40
Soil†			10	53	18	3		101	63	65	70
H. sativum											
Wheat	1.6	35	39	32	44	36	94	82	61	45	43
	4.7	31	30	28	40	34	73	85	79	68	52
Oat	1.6	30	44	32	37	30	85	73	63	46	46
	4.7	22	30	33	40	21	91	103	72	53	46
Barley	1.6	44	41	29	45	22	88	89	70	51	48
	4.7	26	28	31	47	18	99	108	78	57	47
Soil*		33	38	29	42	14	101	83	68	46	47
Soil†			31	24	24	19		101	90	97	66
F. culmorum											
Wheat	1.6	21	29	36	45	32	112	81	82	50	43
	4.7	31	22	33	37	25	80	88	85	60	58
Oat	1.6	16	21	25	32	14	74	92	89	53	47
	4.7	17	20	16	25	11	89	102	77	49	56
Barley	1.6	49	38	30	34	24	95	96	72	56	44
	4.7	27	35	29	35	20	107	105	63	60	50
Soil*		16	36	26	44	26	117	88	70	42	41
Soil†			6	11	22	4		97	82	68	57
Uninoculated											
Wheat	1.6	27	36	25	44	28	103	50	60	47	40
	4.7	43	29	22	29	22	67	69	79	63	45
Oat	1.6	10	19	17	26	16	85	57	72	45	43
	4.7	18	15	14	19	23	103	83	78	62	45
Barley	1.6	30	23	21	33	35	83	74	66	50	43
	4.7	23	22	19	30	18	99	79	91	46	44
Soil*		22	29	25	43	27	103	62	56	43	42
Soil†			5	9	10	5		100	95	67	70

* *Permanent control.*
† *Control freshly prepared for each planting.*

were 26, 30, and 30%, respectively, indicating that, during decomposition, wheat straw rather favours disease, and that decomposing oat straw tends to check it. Also, there was some evidence of a trend toward less disease at the 4.7% concentration of straw than at 1.6%. Finally, it was again evident that a relatively small amount (10 gm.) of inoculum added to a natural soil was, under the conditions of these experiments, usually only slightly effective on the first crop (Fig. 2 E and F, and Table V).

Discussion

In these experiments, where a small quantity of inoculum was added to the pots, disease development was relatively light on the first crop of seedlings and, as is usual, it was definitely less on the second and third crops. It was never as severe as that which usually occurs in a freshly steam-sterilized, inoculated soil. There was some tendency for an increase of disease beginning with the fourth crop from the originally inoculated series. This trend was not consistent. Also, beginning with the second planting, the increase of disease in the various control units, which were not artificially infested, was almost identical with that of the corresponding units originally infested artificially. Thus, it would appear that the increase during a year or so of a pathogen in a given soil, whether in field or greenhouse, would depend mostly on the initial natural infestation, assuming, of course that other conditions were favourable. In other words, if the bio–physico-chemical state of a given soil were not favourable for a natural increase of the pathogen, a large quantity of artificial inoculum would be no more effective than a relatively small amount.

There was significantly less disease on the seedlings grown on oat-straw compost than on those from either of the other two composts. Also, in general, the composts of barley-straw seemed slightly less conducive to disease than those of wheat straw. The results mentioned may be best explained by assuming that the dominant microflora of the oat-straw composts were somewhat antagonistic to the pathogens, or characteristically different from that of the other composts (23).

There was some evidence that the concentration of straw in a compost affected the severity of disease, but this effect was not consistent enough in different plantings to warrant a conclusion. If each kind of straw has a characteristic microflora associated with its decomposition, the final effect on the pathogen might depend more on the degree of antagonism present than on certain differences in its concentration. This theory is supported by the conclusion of Waksman and Hutchings (26) that wheat straw is more resistant to decomposition than oat straw, and by the work of Martin (12) and Millar *et al.* (13), who concluded that the maximum rate of decomposition of a given plant material depends upon the presence of a specific microflora.

This study provides some interesting comparisons on the rate of increase of the three pathogens in the various inoculated and uninoculated series. First, *O. graminis* produced disease only on the first crop following inoculation,

and then seemed to disappear completely, regardless of the presence or absence of decomposing wheat, barley, or oat straw. In contrast is the interesting fact that, starting with a slight natural infestation of a summerfallowed field, this pathogen is known to increase sufficiently to cause fairly severe damage to the second and third successive crops of wheat. Apparently the conditions necessary for the rapid increase of *O. graminis* are provided by successive crops of wheat in the field, but not in pot culture in the greenhouse.

H. sativum and *F. culmorum* are similar to *O. graminis* with respect to rapid vitiation of artificial inoculum, whether added to the soil in either field or greenhouse culture, but different in that both tend to increase in greenhouse culture with successive cropping to wheat. Such increase does not seem to depend upon the original inoculum added, nor is it appreciably affected by the presence or absence of decaying wheat, oat, or barley straw in the soil.

Plant vigour was used as an aid in estimating the relative effect of the different kinds of straw composts on disease. The height of the plants was not considered as reliable an index as dry weight, for during the winter months, despite supplementary artificial illumination, the plants were tall in relation to their weight, whereas during autumn and spring, under normal sunlight, the relation between height and weight was close. Growth was significantly suppressed in the first planting on the three kinds of composts. However, in the second planting this inhibition was replaced by a stimulatory effect, which usually reached a maximum by the fourth planting. After this time plant vigour gradually decreased, even though light conditions were more favourable. Evidently this latter decrease in vigour was due to natural reduction of available plant nutrients.

Plants grown on wheat-straw composts were, in general, the least vigorous throughout the study. The most vigorous plants were produced on the barley-straw composts. Growth on oat-straw composts varied. In 1935, when the straw was immature, the plants were quite definitely lacking in vigour and rather chlorotic, but in 1936 and in 1938 they were better than those on wheat straw, and only slightly less vigorous than the seedlings on the barley-straw composts. With regard to the relation of the amount of straw in a compost to vigour, it was generally apparent that when a particular kind of straw reduced growth, it was directly proportional to the amount added. Thus, in the case of wheat straw, least growth occurred at the highest concentration, while for barley straw the opposite was true. In order to explain the situation just mentioned, one must assume that it was due to one or more factors, namely, the chemical composition of the straw, the release of toxins, rate of decomposition, the kinds of micro-organisms involved, and the C/N ratio established in the different composts.

With regard to toxins, Collison and Conn (5) found that products toxic to plants were released from decomposing wheat or barley straw. Similar evidence is supplied by Waksman (24). As for the effect of the chemical constitution of certain crop debris on decomposition, Hutchings and Martin (11) found that this was more important than the C/N ratio established in

the soil. Further, according to data secured by the present study, indications were that the difference in nitrate nitrogen content of the three kinds of straw were not directly proportional to the differences observed in plant vigour. However, one might assume that the complete chemical complex of a given organic material is often apt to be of greater importance to plant growth than a single chemical constituent of it.

Thus, concluding this discussion, it appears that the observed differences in persistence and virulence of the pathogen, and in plant vigour, were caused mainly by differences in the microflora associated with the decomposition of each kind of compost.

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THE INTERACTION OF HIGHER PLANTS AND SOIL MICRO-ORGANISMS

I. MICROBIAL POPULATION OF RHIZOSPHERE OF SEEDLINGS OF CERTAIN CULTIVATED PLANTS[1]

By M. I. Timonin[2]

Abstract

The microbial population in the rhizosphere of wheat, oats, alfalfa, and peas was studied and the relative abundance of different types of micro-organisms recorded. By means of the plating method it was found that bacteria and actinomycetes were 7 to 71 times greater in the rhizosphere than in the soil distant from the roots, whereas fungi were but 0.75 to 3.1 times more numerous.

Different varieties of plants affected the activity of the various groups of soil micro-organisms differently. Thus fungi were more numerous in the rhizosphere of oats, and bacteria in the rhizosphere of alfalfa. Seventeen genera of fungi were represented in isolates from the rhizosphere of seedlings and the soil distant from the roots. However, no marked difference was observed in the types isolated from the rhizosphere of different varieties of seedlings. A fungus isolated from the rhizosphere of alfalfa proved to be a new species and the genus *Spicularia* Persoon was amended to include it.

Introduction

The living part of the soil complex consists of immense numbers of micro-organisms and of roots of higher plants. These two domains of living organisms, using the organo-mineral part of the soil as the medium for their existence, are not only in direct contact but also in a state of continuous interaction. The portion of the soil in which this interaction takes place is called the rhizosphere. It is from the rhizosphere that higher plants obtain their necessary nutrients and it is into the rhizosphere that they excrete by-products of their growth. Owing to the physiological function of the roots, not only are the physical and chemical properties of the soil of the rhizosphere changed, but the microbial activities are greatly affected.

The immense microbial accumulations localized in the near vicinity of the roots may be directly (nodule bacteria, mycorrhizal fungi) or indirectly (e.g., nitrifiers, cellulose decomposers) beneficial to higher plants. However, it should be recognized that the development of different micro-flora in the rhizosphere may be injurious.

Plants in the seedling stage are very sensitive to environmental conditions including direct or indirect attack by soil micro-organisms. The interaction of soil micro-organisms and the seedlings of cultivated plants is therefore of great scientific as well as practical interest. The purpose of this and the following investigations is to throw some light on this interaction and the influence of environmental conditions on it.

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Historical Review

Considerable work on the influence of higher plants on the activity of soil micro-organisms has been recorded in the literature. It can be roughly subdivided into two groups as follows: *(a)* reports concerned with changes of microbial accumulations in cultivated soil owing to the influence of growing crops, and *(b)* reports chiefly concerned with the microbial population of the rhizosphere of cultivated plants.

Caron (3) in 1895 was the first to report that the microbial population of the soil is greatly influenced by the crop grown and the methods of cultivation of the soil. In the same type of soil he found four times as many bacteria under clover as under sugar beets. LeClair (16) and Greaves *et al.* (10) found more organisms in cultivated than in fallow soil. Greaves (9) reported more organisms in the soil under wheat than in the corresponding soil under alfalfa, though Creuzburg (4) reported results contrary to this. Stoklasa (27) obtained different numbers of soil organisms from soil under different crops. Furthermore, his results indicate a positive correlation between the numbers of bacteria in soil under different crops and the evolution of carbon dioxide.

In the middle of the nineteenth century it was already proved (13, 23) that plants through their root systems excrete certain chemical compounds into the surrounding soil. Taking this fact into consideration, Hiltner (11) claimed that the root excretions made the soil around the roots a very favourable medium for the activity of soil micro-organisms. He found greater microbial accumulations in the soil around the roots than elsewhere in the same soil, and to the portion of the soil influenced by the physiological function of the roots he applied the term "rhizosphere".

Hiltner's results concerning the abundance of micro-organisms in the rhizosphere were later confirmed by many workers (8, 12, 21, 22, 30). It has also been shown that the activity of micro-organisms is markedly affected by the degree of plant development. The maximum effect was noticed when the plant was in an advanced stage of vegetative development or in the flowering stage (26). Smith (24) reported stimulative effects of leguminous plants on the number of *B. radiobacter* in the third week after planting. Other workers also presented evidence that different plants exercise a different influence on the activity of soil micro-organisms (25). Krassilnikov *et al.* (14) presented evidence showing that different stages of plant development support different types of soil micro-organisms. Obrazcov (19), however, failed to note any difference in types of micro-organisms in the rhizosphere of different plants. Several other workers (15, 18, 25) reported on the interaction of higher plants and soil micro-organisms; but there is practically no information concerning the influence of the higher plants in the seedling stage on the soil micro-organisms. During the last three decades, the fungous flora of the soil has attracted the attention of several investigators (1, 2, 28, 31, 32). The subject has been studied from various points of view; however, there are practically no records concerning the influence of higher plants on the fungous flora of the rhizosphere.

Material and Methods

A Sassafras sandy loam soil of pH 5.6, and with 4.9% of organic matter, was passed through a $\frac{1}{8}$-in. mesh sieve and placed in the greenhouse in a wooden box, 5 by 2 by 1 ft. Seeds of Kota hard spring wheat, oats, alfalfa, and soybeans were planted. Each variety was sown in a separate row, allowing enough space that roots of individual plants as well as of different varieties would not overlap. Prior to sowing, seeds were surface sterilized in mercuric chloride 1 : 1,000 solution: oats for seven, wheat and peas for five, and alfalfa for two minutes. After sterilization the seeds were washed in sterilized tap water in five consecutive changes. The samples of seedlings and the soil (control) were taken for microbiological analyses 3, 6, 10, and 16 days after planting.

Samples of the soil from the rhizosphere of the seedlings were obtained as follows: the block of soil about 2½ by 2½ by 3 to 5 in. surrounding the seedling roots was cut out and placed in a large porcelain evaporating dish. Different numbers of seedlings were taken at different times of sampling. Thus three days after planting, 3 to 5 seedlings of wheat, oats, and peas, and 10 to 15 seedlings of alfalfa were obtained. In the laboratory the soil blocks were gently crushed with as little tearing of the roots as possible, the seedling carefully removed, and the roots gently shaken. The top at the crown or foot of the seedling was cut off and the roots with the soil attached deposited in a sterile tared Erlenmeyer flask. The flask and contents were weighed again and the weight of roots with the attached soil determined. Assuming that the weight of the attached soil was approximately one-half to one-third (depending on how much soil was attached to the roots) of the total weight, the dilutions for plating were prepared accordingly.

The dilution in the original flask was made 1 : 100 and was shaken by hand for five minutes. Subsequent dilutions were shaken for one minute. When the required dilutions were prepared, the roots were removed by means of forceps from the original flask, the water evaporated on a water bath, and the soil residue dried to constant weight in an oven at 105° C. Thus the weight of moisture-free rhizosphere soil was obtained so that dilution coefficients could be calculated. The dilutions of soil (control) were made on a 10 gm. basis.

The numbers of micro-organisms were estimated by the plating method, counts of suitable dilutions being made on four replicate plates and the average counts recorded. Waksman's (6) peptone-glucose acid agar (pH approx. 3.8 to 4.0) was used for fungi. The numbers of bacteria and actinomycetes were estimated on Waksman's sodium albuminate agar (6) by counting all colonies developing on the plate. All plates were incubated at 28° C., four days for fungi and ten for bacteria and actinomycetes.

For the systematic study of fungi one plate with good distribution of colonies was selected and each individual colony transferred to a slant of the same (non-acidified) medium.

Density of Microbial Population in the Rhizosphere

From the data presented in Tables I and II and in Figs. 1 and 2 it is quite evident that the numbers of microbial counts are larger, except in one case, in the rhizosphere of all four varieties of plants studied, than elsewhere in the same soil. The numerical difference in counts in the rhizosphere and in the soil distant from the roots is clearly shown in the rhizosphere-soil ratio. Counts of bacteria and actinomycetes are from 7 to 71 times greater than in the soil elsewhere. In the case of filamentous fungi, the difference is not as striking, the lowest ratio being 0.75 and the highest 3.1. The data also indicate that each of four varieties of plants influences to a different degree the relative abundance of the various groups of organisms in its rhizosphere. Comparing the counts of colonies obtained from the rhizosphere of the different varieties of the plants studied, oats showed lowest counts in the case of bacteria and actinomycetes, and highest in the case of filamentous fungi in all samples. Alfalfa, however, was higher in bacteria and somewhat intermediate in the case of fungi. As shown in Figs. 1 and 2, the data indicate in general an increase in counts with advancing development of the seedlings.

TABLE I

NUMBERS OF BACTERIA AND ACTINOMYCETES IN THE RHIZOSPHERE AND SOIL DISTANT FROM THE ROOT

Numbers expressed in millions per gram of moisture free soil

Plant	Sample from	1st Sample		2nd Sample		3rd Sample		4th Sample	
		Numbers	$\frac{R}{S}$ ratio	Numbers	$\frac{R}{S}$ ratio	Numbers	$\frac{R}{S}$ ratio	Numbers	$\frac{R}{S}$ ratio
Wheat	Rhizosphere	275.8	12.9	1110.2	49.3	360.7	16.1	523.3	19.1
	Soil	21.3		22.5		28.5		27.3	
Oats	Rhizosphere	230.2	11.9	180.1	8.5	210.5	7.7	312.4	12.8
	Soil	20.7		21.3		27.3		24.5	
Alfalfa	Rhizosphere	287.6	12.9	870.4	42.9	1150.4	46.8	1824.3	71.0
	Soil	22.3		20.4		24.6		25.7	
Peas	Rhizosphere	470.0	22.8	540.1	24.0	800.4	30.3	934.5	37.8
	Soil	20.6		22.5		26.4		24.7	
Average in soil		21.2		21.7		26.7		25.6	

$\frac{R}{S} = \frac{\text{Rhizosphere; soil near the roots}}{\text{Soil distant from the roots (control)}}$

TABLE II

NUMBER OF FILAMENTOUS FUNGI IN THE RHIZOSPHERE AND IN THE SOIL DISTANT FROM THE ROOTS

Numbers expressed per gram of moisture free soil

Plant	Sample from	1st Sampling		2nd Sampling		3rd Sampling		4th Sampling	
		Numbers	R/S ratio	Numbers	R/S ratio	Numbers	R/S ratio	Numbers	R/S ratio
Wheat	Rhizosphere	190,000	1.1	241,000	1.3	320,000	1.9	256,000	1.4
	Soil	175,000		180,000		172,000		172,000	
Oats	Rhizosphere	210,000	1.1	320,000	1.8	415,000	2.5	570,000	3.1
	Soil	185,000		174,000		168,000		186,000	
Alfalfa	Rhizosphere	130,000	0.75	210,000	1.2	231,000	1.3	511,000	3.0
	Soil	174,000		176,000		176,000		169,000	
Peas	Rhizosphere	241,000	1.4	212,000	1.2	272,000	1.5	460,000	2.7
	Soil	169,000		172,000		173,000		173,000	
Average in soil		176,000		176,000		172,000		175,000	

$$\frac{R}{S} = \frac{\text{Numbers in rhizosphere}}{\text{Numbers in soil (control)}}$$

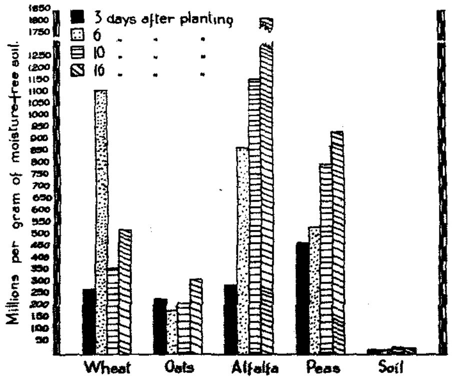

FIG. 1. *Numbers of bacteria and actinomycetes in the rhizosphere and soil distant from the roots.*

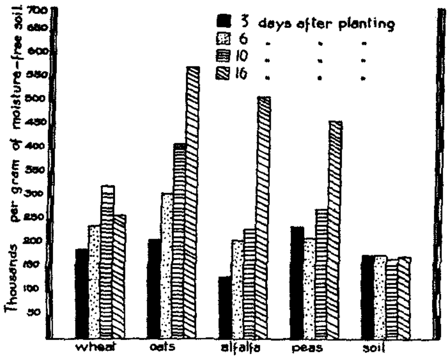

FIG. 2. *Number of fungi in the rhizosphere and soil distant from the roots.*

Qualitative Study of Filamentous Fungi

From Table III it is seen that 16 genera of fungi were recognized in cultures isolated from the rhizosphere and soil distant from the roots. One new species of an emended genus was described.

On examination of the list of genera and species presented in Table III, it is quite evident that there is no significant difference in fungal population in the rhizosphere of the different plants or between the rhizosphere and the soil distant from the roots.

Penicillium, *Trichoderma*, *Fusarium*, and *Coniothyrium* were the prevailing genera in the rhizospheres of all four varieties of plants studied. However, the same genera were also most numerous in the soil distant from the roots. *Phycomycetes* were represented by three genera and were not numerous. *Aspergillus* was represented by only two species, and was not as numerous as *Penicillium*. Species of other genera were obtained so infrequently that they cannot be regarded as characteristic of the different rhizospheres.

An interesting fungus, No. 1121, was isolated from the rhizosphere of alfalfa. It was observed only once on two plates of the same sample, containing respectively two and three colonies of the organism. The conidiophores show a resemblance to the genus *Spicularia* Fuckel. A subculture of this fungus was sent to E. W. Mason, Imperial Mycological Institute, Kew, England. In reply Mr. Mason made the following statement: "The conidiophores and the conidia show a remarkable resemblance to *Spicularia icterus* Fuckel. The original specimen of *Spicularia icterus* is represented at Kew, but I can find no fungus on the leaf. I would rather strongly recommend that the specimen in the herbarium at New York Botanical Garden, Harvard, or Washington be examined."

TABLE III

FREQUENCY OF ISOLATION OF FUNGI IN SOIL NEAR AND DISTANT FROM ROOTS

Fungus	From the rhizosphere of seedlings of				From soil distant from the roots			
	Wheat	Oats	Alfalfa	Peas	First sample	Second sample	Third sample	Fourth sample
Cunninghamella spp.		x		x				
Mucor spp.	x	xx	x	xx		xxx		
Rhizopus nigricans	x	xx	xxx		x			x
Alternaria spp.	x	x					x	
Aspergillus niger	xx						x	
A. versicolor				x		x		
Aspergillus spp.		x			x			
Cephalosporium spp.	x	xx			x			
Cladosporium herbarum	xx	xx	x					
Coniothyrium fuckeli	xx	xx	xxxx	x	x	xx		xxxx
Fusarium spp.	xxxx	xxxx	xxxx	xxx	xxx	xxxx	xxx	xxxx
Gliocladium roseum	xx	xx	x			xx	x	
Mesobotrys spp.			xxx					
Metarrhizium spp.						x		
Monosporium spp.							x	
Penicillium chrysogenum				x		x		
P. intricatum	xx	xx	xx	x		x	xxx	x
P. janthinellum	x	xx		xx	x	x		
P. rugulosum	xx	xxx	xx	x	xx	x	x	xxx
P. tomii		xxx		x			xxx	
P. variabile	x	xx	x		x	x	x	xx
Penicillium spp.	xx	x	xxx	xxx	xxxx	xxx	xx	x
Phoma spp.	x	x			x	x		
Trichoderma album	x							
T. lignorum	xxx		xxxx		xx	x	x	xx
T. koningi		xx	xxx			x		xx
Trichoderma spp.	xxx	xx	x		x		x	x

x = Frequency of organisms isolated from four samples.

The specimen labelled *Spicularia icterus* Fuckel was carefully examined at New York Botanical Gardens by the author, but no fungus was found on the specimen. Dr. H. Linder, curator of the Herbarium of Cryptogamic Botany, Harvard University, and Mr. J. A. Stevenson, Bureau of Plant Industry, Washington, kindly examined the material at their disposal, but the results were also negative. Dr. Linder in his reply stated: "I have just made an examination of No. 2212 of the Fungi Rhenani in a search for *Spicularia icterus* but the only thing found in the meager collection that resembles that specimen is, I am positive, a species of *Botrytis*." Mr. Stevenson also found only a few spherical hyaline conidia approximately 4μ in diameter. From the evidence presented it is quite apparent that there is none of Fuckel's material and no specimen of *Spicularia* Fuckel from any other sources available on this continent or at the Imperial Mycological Institute, Kew, England, with which the fungus No. 1121 could be compared. Therefore the only possible comparison is with Fuckel's description of *S. icterus*.

The generic name *Spicularia* was first established by Persoon (20), but later all his species were transferred by Fries (5) to the genus *Botrytis*. The

genus *Spicularia* Persoon thereupon disappeared until it was brought into being again by Fuckel to take the species *S. icterus* Fuckel. Owing to the fact that spores of *S. icterus* were described as "simplicibus (?)" and "candidis" with hyaline mycelium, Lindau (17) included it in Moniliaceae of the Cephalosporieae subsection with the following criticism, "Fuckel accepts that the fungus causes a disease of grape known by the name yellows. What the author said certainly does not represent the cause of the systematic disease. For example he also introduces the Phyloxera into this discussion. The present day conception of grape yellows indicates that our fungus has no relation to it. It may be traced to soil or climatic conditions. *Spicularia* may then be regarded as a saprophyte upon dry leafspots. The rare occurrence of this fungus apparently substantiates this assumption, because it seems that since Fuckel's time the fungus has never been found again. Fuckel's drawing which has been reproduced herewith does not appear to me to be entirely correct."

Taking into consideration Lindau's criticism of Fuckel's description of S. icterus and the fact that there is no material available with which Fuckel's description of *S. icterus* or the fungus No. 1121 could be compared, and since the fungus No. 1121 closely resembles Fuckel's *Spicularia*, we therefore retain the generic name *Spicularia* and the fungus No. 1121 may constitute the type species, unless Fuckel's fungus be collected again and proved to have uniseptate conidia.

Spicularia (Persoon) Em. Nov.

Hyphae creeping, septate, branched, hyaline, or slightly coloured. Conidiophores erect, unbranched, septate, dark olive-brown, bearing at the tip a whorl of club-shaped sterigmata-like cells. Sterigmata are unbranched, nonseptate, irregularly swollen at the apex; apex papillate. Conidia are borne singly on each papilla, uniseptate, dark brown, smooth.

Spicularia terrestris n. sp.

Colonies on potato dextrose agar slow spreading, consisting mostly of submerged mycelium and aerial conidiophores; surface velvety in appearance, dark coffee-brown to fuscous; (Ridgway, Plate XLVI); reverse black-brown. In rapidly growing colonies the hyphae near the margin showing a yellow tint. Mycelium branched, sparsely septate with numerous terminal and intercalary dark olive-brown chlamydospores. Conidiophores (Fig. 3) arise from submerged or aerial mycelium, at the point of origin produces 1 to 2 rhizoid-like hyphae; erect, slightly tapering toward apex, unbranched, septate, dark brown up to upper septum, smooth, up to 450μ long. At the apex, which is slightly inflated, bearing a whorl (up to 12) of club-shaped sterigmata-like cells. Sterigmata are unbranched, non-septate and irregularly swollen at the apex covered with numerous papillae. Sterigma are 16 to 18μ long, 3 to 4.5μ wide at the base, and the swollen part up to 8μ thick. Conidia borne singly on each papilla, oblong-ovate, two-celled, slightly constricted at the septum; lower cell often bearing remains of papilla; light brown to fuscous, smooth, 6–9 $\times$ 3–5μ.

FIG. 3. *Camera lucida drawings of Spicularia terrestris. (a) Conidiophore, (b and b_1) sterigmata, (c and c_1) conidia, (d) bases of conidiophores with rhizoid-like hyphae, (e) submerged hyphae bearing terminal and intercalary chlamydospores.*

Spicularia (Persoon) Em. nov.

Hyphae repentes, septatae, ramosae, hyalinae vel leviter coloratae. Conidiophorae erectae, haud ramosae, septatae, fusco-olivaceae, verticillum cellularum clavatarum similium sterigmatibus apice ferentes. Sterigmata neque ramosa neque septata, apice papillato inaequaliter tumida. Conidia in quaque papilla singulatim gestata, uniseptata, badia, levia.

Spicularia terrestris n. sp.

Societatibus lente extendentibus in agari mixto cum dextro saccharo ex Solano tuberoso exorto, plerumque constantibus ex submerso mycelio et aeriis conidiophoris; superficie visu velutina, caliginosa vel fusca (Ridgway, Plate XLVI) nigrofusca in latere opposito. Hyphis prope marginem societatum crescentium celeriter luteis. Mycelio ramoso, sparsim septato, chlamydosporis plurimis fusco-olivaceis quum terminalibus tum intercalariis. Conidiophoris (Fig. 3) ex submerso aut aerio mycelio exorientibus, loco originis 1–2 hyphas similes radicibus parientibus, erectis, apicem versus leviter tenuius evadentibus, haud ramosis, septatis, caliginosis usque ad septum superum, levibus, usque ad 450μ longis, apice leviter tumido verticillum (usque ad 12 numero) cellularum clavatarum ad similitudinem sterigmatum accedentium gerentibus. Sterigmatibus haud ramosis, haud septatis, apice irregulariter tumido papillis numerosis tecto. Sterigmatibus 16–18μ longis, 3–4.5 μ base latis, parte tumida usque ad 8μ crassa. Conidiis in quaque papilla singulatim partis, oblongo-ovatis, uniseptatis, septo leviter constrictis, fulvis vel fuscis, levibus 6–9 $\times$ 3–5μ, cellula inferiore reliquias papillae saepe ferente.

Discussion

From the results of this investigation it is quite apparent that seedlings, although only three days old, were already harbouring in their rhizospheres from 11 to 28 times as great a bacterial population as elsewhere in the same soil. In the case of fungi, however, the increase is not as striking. The discrepancies of the plate method for the estimation of soil fungi are well known to soil microbiologists. Considering the fact that fungous colonies developing on an agar plate may originate, not only from spores, but also from bits of mycelium, which latter is harder to disperse or to break during shaking in the preparation of the dilution than spores or conidia, it is quite possible that increase of mycelium in the rhizosphere could be far greater than indicated by the plate method.

A second point of interest is that the duration of the experiment (15 days) is not sufficient to permit the assumption that the seedlings deposited enough organic residue of the root tissue to account for the increase of microbial activity noted. While it is true enough that there is some sloughing of epithelial cells from the growing root tips which may favour microbial development to some extent, it is considered that the excretion of water-soluble chemical compounds by the roots is the chief cause of the increased microbial activity in the rhizosphere. To elucidate these questions further research is necessary.

Acknowledgments

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PRUNUS IN EASTERN CANADA[1]

By Herbert Groh[2] and Harold A. Senn[2]

Abstract

The distribution of *Prunus* species in Eastern Canada is of definite economic significance since these species serve as secondary hosts for leaf hoppers and aphids, which are vectors of important virus diseases.

A key is presented for the determination of the following native and naturalized species: *Prunus spinosa, insititia, domestica, americana, nigra, Persica, pumila, avium, Cerasus, mahaleb, pennsylvanica, serotina, Padus*, and *virginiana*. The detailed distribution of these species is outlined through the citation of herbarium specimens and Canadian Weed Survey records, the ranges of the more important species being mapped.

Cultivated and naturalized species are considered to be of little significance in disease transmittal. In potato-growing regions the choke cherry (*P. virginiana*) which frequents fence-rows is probably the most important species, although the bird cherry (*P. pennsylvanica*) may also be important on light sandy soils.

Introduction

Recent enquiries from entomologists and plant pathologists regarding the distribution and incidence in Eastern Canada of certain wild plums and cherries were the immediate occasion for the preparation of this paper.

It has been represented that plums occurring in the Niagara fruit belt are the hosts of the leafhopper vector of peach virus diseases. Investigations proceeding in the potato-growing districts of Eastern Canada also indicate that *Prunus* species serve as the overwintering hosts of the species of aphid most concerned in the distribution of potato diseases.

Trial tabulations of some of the *Prunus* records available in the Canadian Weed Survey files in the Division of Botany and Plant Pathology were made by Miss Jean C. Burnham, Dominion Entomological Laboratory, Fredericton, N.B. These, together with the subsequent studies here reported, are a definite indication of the value of having an adequate knowledge of the distribution of Canadian plants generally, upon which knowledge many details of agricultural practice have to be based. In view of what had been disclosed the Dominion Botanist urged that all the botanical information available should be properly compiled and placed at the disposal of all workers interested.

The records referred to above were quite fortuitously available for the use suggested, having been secured in the course of weed surveys initiated in 1923 and still continuing. Partly as fence-row or waste land encumbrances, but chiefly because of reputed responsibility for prussic acid poisoning of animals browsing upon the wilted foliage of *Prunus* species, the two most generally common members of the genus, *P. virginiana* L., the choke cherry,

[1] *Manuscript received November 16, 1939.*

Contribution No. 605, Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] *Associate Botanist.*

and *P. pennsylvanica* L.f., bird or wild red cherry, had been admitted to the list of weeds under survey. For other than those two species no such source of information was at hand. Specimens had not ordinarily been collected of either these or other species. A certain amount of information was available in the literature, but it was soon realized that this had to be accepted with a good deal of discrimination, particularly in the case of some species. Taking all things into account it was deemed advisable to assemble and make the widest possible examination of herbarium material. For this purpose the larger and most of the smaller collections in Eastern Canadian and United States institutions were placed freely at our service. Of most species enough material was thus available to give a broad outline, at least, of the desired range limitations within the eastern half of Canada.

In one respect the extensive citations that follow are not all that is to be desired. A rather large proportion of those of most native species are from areas outside of present or likely agricultural settlement, owing to an apparent flair of botanists for collecting in more virgin fields. For the study of incidence here required they tend to misplace emphasis. This is in some contrast with the weed survey records already referred to which were secured largely from within the territory to be served by such a study as this. The two types of data are required to complement each other, even if not equivalent in all respects.

The objection which will be raised in some quarters that sight records, unsupported by specimens, are invalid evidence should not be given undue weight in the case of two species as readily distinguishable in the field as the choke cherry and the bird cherry of Eastern North America. The occasional occurrence of black cherry in the more southerly part of their range can be a source of confusion at times. Beyond our westerly limits, also, eastern and western species of slightly differing appearance would be a problem. Granted that misidentifications in the field can occur and cannot be checked afterwards, it may still be affirmed that the wealth of records here personally secured by careful reconnaissance methods is amply large to minimize any error which may chance to be included. If obviously extra-territorial, a record unsupported by a specimen would be rejected; within the expected range, the most that a chance error can do to the general result is to alter by one in the total the frequency index secured.

The present treatment includes all the native *Prunus* species of Eastern Canada and all the species that have become established as escapes from cultivation. It does not include the species that are cultivated primarily as ornamentals, since no records were discovered to indicate that such species had become naturalized. This should not, however, be interpreted as an indication that such species may not play a part in the overwintering of the insects mentioned above.

Since no key was available for the determination of the group of species within the purview of this study the key presented below was prepared. The more important North American synonymy for each species has been included,

but no attempt has been made to present a complete bibliography, especially of Asiatic and European species.

Methods

Eastern Canada is here taken to mean west to Meridian 92°, which divides the settled belt more equally into halves for mapping than would be the case if the Manitoba-Ontario boundary were used. Exigencies of mapping are also allowed to exclude Newfoundland on the east, for which there are citations in some instances but not survey records.

The ranges of the native species for which no weed survey records were available (*Prunus americana*, *nigra*, *pumila*, and *serotina*) have been mapped from cited specimens on outline maps (see Figs. 1, 2, 3, 4, 5, 8).

Special maps have been prepared for *P. pennsylvanica* (Fig. 7) and *P. virginiana* (Fig. 9) including both citations of herbarium specimens and Weed Survey records. Fig. 6 records all points at which weed surveys were made, thus indicating by comparison with Figs. 7 and 9 the points at which surveys were made but no *Prunus* was found.

Instead of a standard base map an outline map has been fitted to squared paper in an approximation to a Mercator's projection. Latitude and longitude are thus conveniently used, instead of confusing map detail, to locate position. When surveys are repeated at any location, displacement of entry on the map is kept as small as possible and within the correct meridian. Entry of herbarium records is also kept more strictly to meridians than to parallels where crowding occurs.

Material Examined

Specimens were seen from the herbaria designated as follows:

A	Acadia University, Wolfville, N.S.
AA	Arnold Arboretum of Harvard University, Jamaica Plain, Mass.
Can	National Herbarium of Canada, Ottawa, Ont.
Dal	Dalhousie University, Halifax, N.S.
DAO	Division of Botany and Plant Pathology, Science Service, Dominion Department of Agriculture, Ottawa, Ont.
G	Gray Herbarium of Harvard University, Cambridge, Mass.
M	University of Montreal, Montreal, Que.
McG	McGill University, Montreal, Que.
McM	McMaster University, Hamilton, Ont.
MV	Private herbarium of Prof. Marie-Victorin, University of Montreal, Montreal, Que.
NBM	New Brunswick Museum, Saint John, N.B.
OAC	Ontario Agricultural College, Guelph, Ont.
Q	Queen's University, Kingston, Ont.
SteA	Ecole Supérieure d'Agriculture, Ste. Anne de la Pocatière, Que.
T	University of Toronto, Toronto, Ont.

Acknowledgments

To the custodians of these various collections our sincere thanks are extended for the courtesies shown. To others, whether named in these pages or not, who have given information or have helped by interest taken in the project, and to Messrs. W. H. Minshall and M. N. Zinck of this Division, for valued assistance in the preparation of the maps, we are also grateful.

Key to the Native and Naturalized *Prunus* Species of Eastern Canada

A. Leaves conduplicate or convolute in the bud; flowers opening before or at the same time as the appearance of the leaves; drupe sulcate, pubescent or with a bloom. Plums and Peaches .... B.

B. Axillary buds solitary, terminal bud wanting; flowers pedicelled in simple umbel-like clusters, white or greenish-white (very rarely pink); drupe glabrous with a bloom; stone smooth or slightly sculptured. Plums .... C.

C. Leaves convolute in the bud; flowers 1–2 per cluster; stone somewhat sculptured. European and Asiatic species escaped from cultivation in Canada .... D.

D. Low shrub, distinctly spiny; young branchlets pubescent; leaves small, 2–4 cm. long; flowers usually solitary; pedicels glabrous, rarely pubescent; fruit globose, small, usually less than 1 cm. in diameter .... 1. *P. spinosa*

D. Shrub or small tree, unarmed or slightly spiny; young branchlets glabrous or pubescent; leaves larger, 4–10 cm. long; flowers 1–2; pedicels pubescent, rarely glabrous; fruit globose or variable in shape, larger, more than 1 cm. in diameter .... E.

E. Shrub or small tree, sometimes slightly spiny, of dwarf compact habit; young branchlets pubescent; leaves relatively small, 4–8 cm. long, tending to be more ovate, with closely serrate margins; pedicels pubescent; fruit regular, globose or ovoid, usually less than 2–5 cm. in diameter .... 2. *P. insititia*

E. Small tree, unarmed, of erect habit; young branchlets glabrous or rarely slightly pubescent; leaves relatively large, 5–10 cm. long, tending to be more elliptic or obovate, with coarsely irregularly serrate margin; pedicels glabrous, rarely pubescent; fruit variable in shape, large, usually more than 2.5 cm. in diameter .... 3. *P. domestica*

C. Leaves conduplicate in the bud; flowers usually 3 or more per cluster; stone usually smooth. American species .... F.

F. Leaf serrations pointed, acute, bristle tipped; calyx lobes entire, devoid of glands; inner surface of the calyx lobes densely pubescent .... 4. *P. americana*

F. Leaf serrations rounded, obtuse, glandular; calyx lobes serrate, glandular; inner surface of the calyx lobes glabrous or sparsely pubescent . . . . 5. *P. nigra*

B. Axillary buds 3; terminal bud present; flowers subsessile, usually solitary, pink; drupe pubescent (very rarely glabrous); stone deeply sculptured. Peaches . . . . 6. *P. Persica*

A. Leaves conduplicate in the bud; flowers usually opening after the appearance of the leaves; drupe not sulcate, neither with a bloom nor pubescent. Cherries . . . . G.

G. Flowers solitary or few, in umbels or in short, few-flowered racemes; bracts, conspicuous . . . . H.

H. Shrubs; buds 3 in each leaf axil; pedicels usually short. Sand cherry . . . . 7. *P. pumila*

H. Trees or tall shrubs; buds solitary; pedicels usually long . . . . I.

I. Leaves dentate with obtuse teeth; involucral bracts at base of the inflorescence persistent; flowers in umbels. Cultivated Eurasian cherries, escaped in Canada . . . . J.

J. Trees, not suckering from the root; leaves long (up to 15 cm. long), pubescent beneath, at least on the veins; inner bud scales reflexed; inflorescences without leafy bracts; calyx lobes entire; fruit sweet . . . . 8. *P. avium*

J. Trees, suckering from the root; leaves short (up to 7 cm. long), glabrous; inner bud scales erect; inflorescence usually with a few leafy bracts; calyx lobes crenate; fruit sour . . . . 9. *P. Cerasus*

I. Leaves crenate-serrulate with sharp pointed teeth; involucral bracts at the base of the inflorescence deciduous; flowers in umbels or short racemes . . . . K.

K. Leaves sub-orbicular or broad-ovate, crenulate-serrate; flowers in short racemes. 10. *P. mahaleb*

K. Leaves oblong-lanceolate, acuminate or rarely acute at the apex, finely and sharply serrate with incurved teeth; flowers in umbels or corymbs . . . . 11. *P. pennsylvanica*

G. Flowers in elongated racemes with numerous flowers; bracts small . . . L.

L. Leaves serrulate with incurved callous teeth; calyx persistent in fruit . . . . 12. *P. serotina*

L. Leaves sharply serrate with slender teeth; calyx deciduous in fruit . . . . M.

M. Leaves elliptic to obovate or oblong-ovate, rounded or subcordate at the base, sharply serrate; calyx-tube pubescent inside; petals twice as long as the stamens; stone sculptured . . . . 13. *P. Padus*

M. Leaves broad-elliptic to obovate, broad-cuneate to rounded at the base, closely serrulate; calyx-tube glabrous inside; petals less than twice as long as the stamens; stone smooth . . . . 14. *P. virginiana*

SUBGENUS I. *Prunophora* Focke

Section 1. *Euprunus* Koehne

1. *Prunus spinosa* L.

Sp. Pl. 475. 1753; Torr. & Gray, Fl. N. Amer. 1: 408. 1840; Bailey, Cycl. Am. Hort. 1447. fig. 1979. 1901; Hedrick, Plums of N.Y. 42. 1911 (see this for synonymy); Rehder, Man. Cult. Trees and Shrubs, 454. 1927.

Common name: Blackthorn (Sloe).

Distribution: Europe, North Africa, Western Asia; introduced and escaped in Eastern Canada.

Specimens examined:

NOVA SCOTIA: Summerville, *Jack 3490* (G), escaped along roadside; Wolfville, *Cameron*, May 6, 1936 (A).

ONTARIO: Galt, *Herriott*, May 30, 1909 (G, Can); Pelee Island, *Macoun*, July 27, 1892 (Can).

2. *Prunus insititia* L.

Cent. Pl. 1: 12. 1755; Amoen. Acad. 4: 273. 1759, non Walt. Fl. Carol. 146. 1788; Robins. & Fern. in Gray's Man. ed. 7, 498. 1908; Hedrick, Plums of N.Y. 34. 1911 (see this for synonymy); Britt. & Brown, Illus. Fl. ed. 2, 2: 326. 1913; Rehder, Man. Cult. Trees and Shrubs, 454. 1927.

Prunus spinosa insititia (L.) A. Gray, Man. ed. 2, 112. 1856.

Common name: Bullace (Damson).

Distribution: Western Asia, Europe; introduced and escaped in Eastern Canada.

Specimens examined:

QUEBEC: Ste. Anne de la Pocatière, *Campagna 8036* (SteA), dry sand; Berthier-en-bas, *Marie-Victorin, Rolland-Germain & Jacques 33194* (AA, MV, M).

3. *Prunus domestica* L.

Sp. Pl. 475. 1753; Bailey, Cycl. Am. Hort. 1447. 1901, in part; Hedrick, Plums of N.Y. 12. 1911 (see this for synonymy); Rehder, Man. Cult. Trees and Shrubs, 455. 1927; Marie-Victorin, Fl. Laurent. 320. 1935.

Common name: Garden plum.

Distribution: Western Asia and Europe; cultivated and escaped in Eastern Canada.

Specimens examined:

NOVA SCOTIA: Halifax, *Jack 3238, 3649* (AA), naturalized; Pictou, *Jack 784* (AA) naturalized, common.

Section 2. *Prunocerasus* Koehne

1. ***Prunus americana*** Marshall,

Arb. Am. 111. 1785; Torr. & Gray, Fl. N. Amer. 1: 407. 1840, *in part*; Torr. Fl. N.Y. 1: 194. 1843, *in part*; Sargent, Silva N. Amer. 4: 19. pl. 150. 1892 (see this for full synonymy); Bailey, Cycl. Am. Hort. 1448. 1901; Robins. & Fern. in Gray's Man. ed. 7, 499. 1908; Hedrick, Plums of N.Y. 56. 1911; Britt. & Brown, Ill. Fl. ed. 2, 2: 323. 1913; Wight, Nat. Am. Sp. Prunus 24. 1915 (U.S. Dept. Agr. Bull. 179); Rehder, Man. Cult. Trees and Shrubs, 458. 1927.

Prunus ignotus Nelson, Bot. Gaz. 42: 53. 1906.

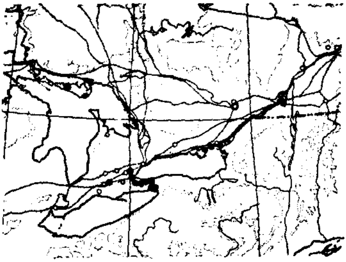


FIG. 1. *Range of Prunus americana Marsh., Wild plum.*

Common name: Wild plum.

Distribution: Eastern and Central United States and southern Ontario; extending into Canada again in southern Manitoba. See Fig. 1.

Specimens examined:

QUEBEC: Caughnawaga, *Jack*, July 30, 1900, Aug. 26, 1908 (AA), probably escaped from plantings.

ONTARIO: Guelph, *Simmons* May 30, 1935 (OAC), hedgerows, *in litt.* reported as escape; (?Hamilton), *Dickson*, May 2, 1895 (McM); Lincoln Co., *McCalla 567* (US); Queenston, *Scott*, Aug. 6, 1902 (Can), *Putman & Armstrong*, May, 1938 (DAO), near Niagara, *Macoun*, May 11, 1901 (Can); Niagara River between Queenston and Niagara-on-the-Lake, *Putman & Armstrong*, May, 1938 (DAO); Chautauqua Park, Niagara-on-the-Lake, *Putman & Armstrong*, May, 1938 (DAO); Jordan Station, *Putman & Armstrong*, May, 1938 (DAO); St. David's, *Putman & Armstrong*, May, 1938 (DAO); Rockway, *Putman & Armstrong*, May, 1938 (DAO); Power Glen, De Cew Falls, *Putman & Armstrong*, May, 1938 (DAO); London, *Burgess*, May 24, 1885 (McG), *Balkwill*, Sept. 9, 1885 (McG).

Mr. W. R. Leslie, Superintendent, Experimental Station, Morden, Man., who has studied the wild plums in Manitoba, in a letter dated December 16, 1938, expresses the view that *P. americana* occurs in the limestone areas, and

P. nigra in the granitic areas to the east and north, although they intermingle along the Red River, especially at Winnipeg. In southern Ontario *P. americana* ranges far short of limestone limits.

5. ***Prunus nigra*** Ait.

Hort. Kew. 2: 165. 1789; Sargent, Silva N. Amer. 4: 15. pl. 149. 1892 (see this for full synonymy); Small, Bull. Torr. Bot. Club, 21: 301. 1894; Robins. & Fern. in Gray's Man. ed. 7, 499. 1908; Hedrick, Plums of N.Y. 69. 1911; Britt. & Brown, Ill. Fl. ed. 2, 2: 323. 1913; Wight, Nat. Am. Sp. Prunus, 21. 1915 (U.S. Dept. Agr. Bull. 179); Sargent, Man. Trees N. Amer. ed. 2, 560. 1922; Rehder, Man. Cult. Trees and Shrubs, 458. 1927; Marie-Victorin, Fl. Laurent. 320, 1935.

Prunus americana var. *nigra* Waugh, Vt. Agr. Exp. Sta. Bull. 53: 60. 1896; Vt. Agr. Exp. Sta. Ann. Rep. 10: 102. 1897; Bailey, Cycl. Am. Hort. 1449. 1901.

Prunus americana American authors, in part.

Common name: Canada plum.

Distribution: Central and northeastern Canada and United States; as far west as the Riding Mts. in Manitoba. See Fig. 2.

Specimens examined:

NOVA SCOTIA: Lansdowne, *Jack 3705* (AA, G), roadside thicket; Wolfville, *Perry*, May 21, 1912 (A); Gaspereau Valley, *McLellan*, June 1, 1939 (DAO).

NEW BRUNSWICK: St. John, *Hay*, June, 1897 (G).

QUEBEC: Quebec, Plains of Abraham, *Michel 1638* (M); Ancienne Lorette, *Marie-Victorin 15535* (M, MV); Lake Memphremagog, Georgeville to Fitch Bay, *Churchill*, Aug. 16, 1903 (G), roadside; Lake Memphremagog, near Oliver Corner, *Churchill*, Aug. 22, 1919 (G), roadside; Pointe du Lac, *Stanislas 444* (M); St. Laurent, *Adrien 20911* (M) *1080* (M, MV); Ahuntsic, *Masson*, May 16, 1925 (M); Ste. Julie de Vercheres, *Marie-Victorin 28636* (M); Cartierville, *Adrien*, May 22, 1925 (M, MV); Ile Bizard, *Adrien, 1714* (M, MV); Montreal, *Robert 22* (M); Longueuil, *Marie-Victorin 189, 4330, 9508, 2093* (MV), *3400* (US), *Marie-Victorin & Rolland-Germain 45203* (M, G), *Rolland-Germain 29420, 29465* (M, MV); Mount Royal, Montreal, *Marie-Victorin 34180* (M, MV); Caughnawaga, *Jack*, May 20, 1900 (AA), *Woodson & Anderson*, Sept. 18, 1931 (G), woodlands, hedgerows; Chateauguay, *Jack 3966* (US); Vaudreuil, *Jack*, Sept. 23, 1913 (AA); Hull, *Harrington*, May 19, 1906, May 23, 1908 (Can); Fairy Lake (near Hull), *E. G. Anderson*, no date (OAC); Farm Point, *Macoun 87170* (Can).

ONTARIO: Summerstown, *Jack*, May 24, 1914 (AA); Cornwall, *Jack*, May 28, 1913, Sept. 24, 1913 (AA); 2 mi. east of Iroquois, *Minshall 1215* (DAO); Ottawa, *Ami*, May, 1879 (McG), *Fletcher*, April 30, 1878, dry woods at Billings Bridge, May 24, 1906 (DAO), *Scott*, May 24, 1890 (OAC), *Macoun*, Aug. 4, 1894 (Can), Billings Bridge, *J. M. Macoun*, May 14, 1891 (Can), Beechwood, *Groh*, April 30, 1910 (DAO), *Minshall*, May 15, 1934 (DAO), Dow's Swamp; Carlsbad Springs, *Macoun*, June 6, 1911 (Can); Stittsville, *Macoun*, May 8, 1903, (Can); Gananoque, *Fowler*, Aug. 18,

1887 (Q); Kingston, *Fowler*, May 23, 1884, May 16, 1900 (Q), May 29, 1905, May 12, 1896, May 7, 1894, May 11, 1901 (US), *Millman*, Sept. 9, 1885 (T, McG); Belleville, *Macoun*, May 24, 1878 (T, Can); Hastings Co., *Macoun*, May 4, 1870 (McG); Toronto, *Scott*, May 14, 1898 (Can), in Don Valley; Arkell, *Bot. Dept., O.A.C.*, May 14, 1931 (OAC); Preston, *Groh*, Dec. 30, 1938 (DAO); Toronto, Bathurst St., near Eglinton, *R. W. Smith*, June 2 (McM); Queenston, *Scott*, Aug. 6, 1902 (T); Niagara Falls, *Macoun*, May 13, 1901 (Can); London, *Millman*, April 30, 1879 (T), *Burgess*, June 21, 1879 (McG); Aylmer, *R. T. Anderson*, May 8, 1898 (T), woods and river banks; Opeongo Lake, Algonquin Park, *Macoun*, Aug. 17, 1900 (T); 51-mile post, District of Thunder Bay, *Macinnis*, Aug. 1900 (Can).

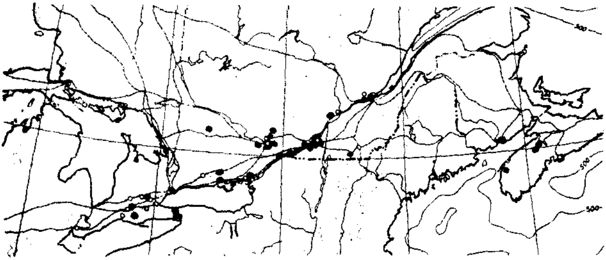


FIG. 2. *Range of Prunus nigra Ait., Canada plum.*

SUBGENUS II. *Amygdalus* Focke

6. *Prunus Persica* (L.) Batsch.

Beytr. Entw. (Pragm.) Gesch. Naturw. 30. 1801*; Bailey, Cycl. Am. Hort. 1457. 1901; Robins. & Fern. in Gray's Man. ed. 7, 499. 1908; Hedrick, Peaches of N.Y. 77. 1917 (which see for full synonymy); Rehder, Man. Cult. Trees and Shrubs, 462. 1927.

Amygdalus Persica L. Sp. Pl. 472. 1753; Britt. & Brown, Illus. Fl. ed. 2, 2: 330. 1913.

Persica vulgaris Mill. Gard. Dict. ed. 8, No. 1. 1768.

Common name: Peach.

Distribution: China, cultivated and locally escaped in southern Ontario.

Specimens examined:

ONTARIO: Queenston, *Scott*, May 18, 1901 (T); Lake Erie, Point Pelee National Park, *Senn 1044* (DAO), escaped along sandy beach.

This is to be expected as an escape in the peach belt, and is not unknown beyond it. A small tree growing on the southern border of woods near Preston, Ontario, about 1915, was found bearing fruit one year.

* *See Junger in Botan. Z. 36: 426. 1878, Koehne in Mitt. Deutsch. Dendr. Ges. 26: 69. 1917.*

Subgenus III. *Cerasus* Pers.

Section I. *Microcerasus* Webb

7. *Prunus pumila* L.

Mant. 75. 1767; Bailey, Cycl. Am. Hort. 1450. 1901; Robins. & Fern. in Gray's Man. ed. 7, 499. 1908; Britt. & Brown, Ill. Fl. ed. 2, 2: 326. 1913; Wight, Nat. Am. Sp. Prunus 65. 1915 (U.S. Dept. Agr. Bull. 179); Hedrick, Cherries of N.Y. 34. 1915 (*in part*); Fernald in Rhodora, 25: 72. 1923; Rehder, Man. Cult. Trees and Shrubs, 466. 1927.

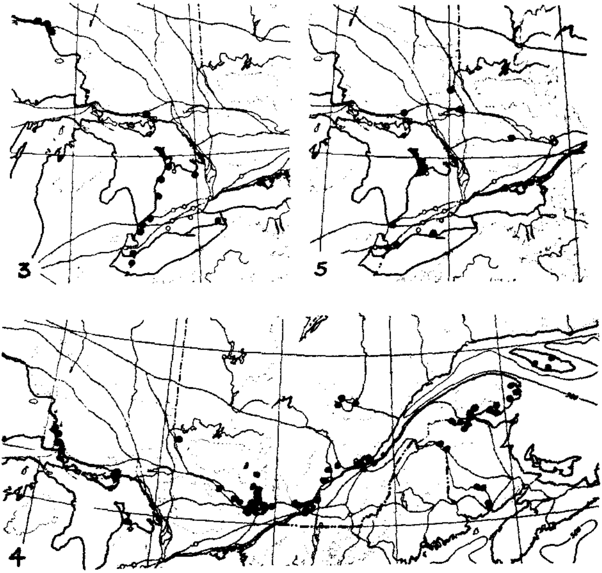
Cerasus canadensis Mill. Gard. Dict. ed. 8. 1768.

Cerasus glauca Moench, Meth. 672. 1794.

Cerasus pumila (L.) Michx. Fl. Bor.-Am. 1: 286. 1803 as to name-bringing synonym only.

Common name: Sand cherry.

Distribution: Eastern Canada and northeastern United States. See Figs. 3, 4, and 5.



FIGS. 3, 4, 5. *Range of Prunus pumila L., Sand cherry. 3. P. pumila L. var. typica. 4. P. pumila L. var. depressa (Pursh) Bean. 5. P. pumila L. var. susquehanae (Willd.) Jaeg.*

We are unable to follow Fernald (Rhodora, 25: 69. 1923) in recognizing as distinct species the three sand cherries of Eastern Canada which have been known as *Prunus pumila* L., *P. depressa* Pursh, and *P. susquehanae* Willd. (*P. cuneata* Raf.). These entities show a complex series of intergradations in the morphological characters which Fernald uses to separate them. In habit the first and last are essentially erect except that *P. pumila* in age tends to become decumbent at the base. *P. depressa* is as a rule, low and spreading. There is considerable intergradation in leaf shape. The leaves of the first two entities are characteristically more elliptical with acute or acuminate apices while the leaves of the latter are more obovate and obtuse. However the lower leaves of *P. depressa* are frequently obovate obtuse and almost indistinguishable from *P. susquehanae*. Wight (Nat. Amer. Sp. *Prunus*, 67. 1915, U.S. Dept. Agr. Bull. 179) while regarding *P. pumila* (including *P. depressa*) and *P. susquehanae* (*P. cuneata*) distinct as regards habit, says "Even mature foliage (of *P. susquehanae*) sometimes approaches *P. pumila* so closely as to render separation difficult, though they are usually much broader toward the base than in the former species."

Fernald (l.c.) placed considerable emphasis on the edibility of the fruit of *P. depressa* in contrast to the small unsatisfactory fruits of *P. pumila*. Yet Bailey (Nat. Dwarf Cherries, Cornell Univ. Agr. Exp. Sta. Bull. 70. 260. 1894) writes of the Great Lakes plant, *P. pumila*:

"This sand cherry is variable in its wild state, especially in its fruit. As a rule, the fruit is small and very sour and scarcely edible, but now and then one comes upon a bush which has fruit of pleasant flavour and as large as small Early Richmond cherries."

The three entities have a somewhat distinct distribution (see Figs. 3, 4, 5), *P. pumila* being centered about the Great Lakes, *P. depressa*, being eastern and northern, and *P. susquehanae* ranging from Pennsylvania across New England and north to Lake Superior. Additional collections seem to be rapidly destroying any supposedly definite boundaries between these forms.

Consequently since there is considerable intergradation in morphological characters and not too sharply delimited geographical ranges we consider that these sand cherries should be regarded as varieties of a polymorphous species rather than as distinct species.

Key to the Varieties of *P. pumila*

A. Shrub, erect or prostrate; leaves usually elliptic to oblanceolate-spatulate, usually acute, relatively thin.

B. Shrub erect (reaching 1.5–2 m. in height) in age decumbent at the base; leaves elliptic, narrow, rarely tending to be oblanceolate-spatulate, acuminate or acute, cuneate at the base, with close sharp divergent serrations; fruit usually small, scarcely edible. Great Lakes..1. var. *typica*

B. Shrub prostrate (rarely over 0.3 m. in height); leaves broader, oblanceolate-spatulate, usually acuminate or acute, but the lower leaves occasionally obtuse, serrations tending to be more crenate; fruit larger, edible. Eastern and northern regions..............2. var. *depressa*

A. Shrub, erect; leaves obovate-spatulate, usually short, obtuse, thicker. Ontario....................................3. var. *susquehanae*

Prunus pumila L. var. *typica*

Specimens examined:

ONTARIO: Wellington, *Fowler*, Sept. 3, 1902 (G); Point Abino, Lake Erie, *Coville*, Aug. 23, 1886 (US), *Keller*, July 11, 1925 (US); Pt. Pelee, *Macoun*, June 29, 1882 (US, Can), *Burgess*, June 30, 1882 (DAO, T), July 1, 1882 (McG); Pelee Island, *Macoun*, July 27, 1892 (Can); Point Edward, *Macoun*, *June 15, 1901, (G, Can); Forest, Howitt, June 11, 1914 (OAC)*; near Sarnia, *Dodge*, May 2, June 19, 1893 (Can); sandy places near Pt. Frank, *Baker* (OAC); Wasaga Beach, Simcoe Co., *Marie-Victorin, Rolland-Germain* & *Meilleur 45106* (M, T); Johnston Harbour, Lake Huron, *Macoun*, Aug. 24, 1901 (G); Stokes Bay, *Krotkov 9148* (T); Big Bay, Tobermory, *Krotkov 7533* (T,G) *7534* (T); Oliphant, Lake Huron, *Breed, Jeffrey, Stebbins, et al. 166* (G); Georgian Bay Biological Station, no data (Can); Cloche Bluff, McGregor Bay, north side of Georgian Bay, *Coville*, July 25, 1915 (US); Lake Superior, *Loring*, no date (G); north shore of Lake Superior, *Macoun*, 1869 (US); Peninsular Harbour, north shore of Lake Superior, *Miller*, Sept. 26, 1896 (G).

7a. *Prunus pumila* L. var. *depressa* (Pursh) Bean,

Trees and Shrubs Hardy in the Br. Isles 2: 249. 1914; Rehder, Man. Cult. Trees and Shrubs 466. 1927.

Prunus depressa Pursh. Fl. Am. Sept. 1: 332. 1814; Fernald in Rhodora, 25: 73. 1923; Marie-Victorin, Fl. Laurent. 320. 1935.

Cerasus pumila Michx. Fl. Bor. Am. 1: 286. 1803, as to shrub described.

Prunus pumila Torr. Fl. N.Y. 1: 195. 1843, non Linn.

Specimens examined:

NEW BRUNSWICK: Drury's Cove, *Hay*, June 15, 1878 (NBM); Grand Lake, *Hay*, July 1, 1876 (Can, Q), another specimen of this collection under the name *pumila*, in Acadia University Herbarium, was not seen by us; Goat Island, Grand Lake, *Fowler*, Sept. 17, 1879 (Q); shores of Upper Restigouche, *Hay*, July 28, 1896 (NBM); Tom's Island, Restigouche River, *Hay*, July 30, 1896 (G); St. John River between Edmundston and Grand Falls, *Hay*, July 19, 1879 (NBM).

QUEBEC: Rivière au Saumon, *Marie-Victorin, Rolland-Germain & Louis-Marie 20888* (MV); Rivière de Brig, Anticosti, *Macoun*, Aug. 10, 1883 (Can); Rivière McKane, Anticosti Id., *Marie-Victorin & Rolland-Germain 27366* (M, MV); Ile à Deux Têtes, L'Estuaire des Saint Laurent, *Rousseau 24658* (US, Can, M, MV); Grand River, Gaspé Co., *Richards*, June 20–July 10, 1903 (G); Mt. St. Pierre, Gaspé Co., *Pease & Goodale*, July 13,

1932 (G), *Fernald, Weatherby & Stebbins 2453* (G, Can, M); Gaspé, *Macoun*, 1882 (Can); Rivière Bonaventure, *Marie-Victorin, Rolland-Germain & Jacques 33263* (M, MV), *45053* (M, Can); Bonaventure River between Baldé and Baie de Chaleur, *Collins, Fernald & Pease, 5829, 5830* (G); Rivière Bonaventure, *Campagna, Arsenault & Bauville*, Aug. 12, 1931 (SteA); Matapedia, *Fernald*, June 28, 1904 (G), *Marie-Victorin 28683* (M, MV); Milnekek, Matapedia River, *Rousseau & Bonin 32039* (M, MV), *32083* (M, US, Can); Restigouche River, 20 mi. below Patapedia River, *Rousseau & Bonin 32218* (Can, M); Junction Restigouche and Patapedia Rivers, *Rousseau & Bonin 32155* (M, MV, Can, US); Lake St. John, *Jack*, Aug. 23, 1895 (AA); Grand Discharge, Lake St. John, *Kennedy*, July 22, 1892 (G), *Marie-Victorin, Rolland-Germain & Meilleur 43031* (M), *LV*, Aug. 1921 (M); Pointe Bleue, Lac St. Jean, *Marie-Victorin 15539* (US, M, MV); Pointe Sud-Ouest de l'Ile d'Orleans, *Marie-Victorin 15538* (AA, US, M, MV); Ste. Petronille, Ile d'Orleans, *Marie-Victorin, Rolland-Germain & Meilleur 45026* (M); St.-Lambert de Laizon, Levis Co., *Marie-Victorin, Rolland-Germain & Meilleur 30535* (M); Pont Rouge, *Marie-Anselme*, Aug. 21, 1938 (DAO); St. Georges, Beauce Co., *Marie-Victorin, Rolland-Germain & Meilleur 43342* (M); Pointe-au-Lac, St. Maurice Co., *Stanislas 445, 446* (M); Shawinigan Falls on St. Maurice River, *Chamberlain & Knowlton*, Aug. 1, 1923 (G); St. Genevieve, Anse à l'Orme, *Adrien*, July 29, 1927 (M, MV); Contrecoeur, Vercheres Co., *Marie-Victorin & Rolland-Germain 29212* (M, MV); Portage de Gres River, Montreal, *Holmes*, July 10, 1882 (McG); between Longueuil and Isle Ste.-Helene, *Marie-Victorin 8340* (MV); Ste. Rose, Laval Co., *Marie-Victorin & Rolland-Germain 43741* (M); Oka, Lake of Two Mountains, collector not stated, 1909, *Marie-Victorin*, July, 1914 (MV); vicinity of Ottawa, *Marie-Victorin, 9130* (M), *10114* (M, MV); Ottawa River, *Rolland 55* (M, MV); vicinity of Ottawa, *Rolland, 7159* (MV); Brigham's Creek, Hull, *Macoun*, Sept. 11, 1894 (Can); island at Chelsea, *Macoun*, July 27, 1911 (Can), approaching *susquehanae;* Kirk's Ferry, *Harrington*, May 20, 1906, July 5, 1908 (Can); Paugan Falls, *Macoun*, Sept. 3, 1894 (Can); Ile des Allumettes, *Marie-Victorin, Rolland-Germain & Meilleur 44946* (M); Kazabazua, *Dore*, June 2, 1932 (DAO); Norway Bay, *Adams*, July 23, 1917 (DAO); Pointe au Vent, Lake Temiscaming, *Marie-Victorin 8285* (AA, MV).

ONTARIO: Ottawa, Rockcliffe Park, *Macoun*, May 26, 1903, (Can, DAO), May 18, 1911 (Can), Chaudière Islands, *Fletcher*, June 3, 1883 (McG, DAO), *Scott*, June 6, 1890 (OAC), May 24, 1891, June 6, 1892 (T), Beechwood, *Scott*, May 24, 1893, May 24, 1898 (T), near Lemieux Island, *Minshall*, May 27, 1937 (DAO); Constance Bay, *Gröh*, May 13, 1930, *Minshall*, May 17, 1934, *Adams*, June 12, 1938 (DAO); Chalk River, *Macoun*, June 30, 1884 (Can, McG); Howdenvale, Bruce Co., *Watson 2942* (US, T); Great Cloche Island, Manitoulin District, *Fernald & Pease* 3398 (G, T); Pancake Bay, *Taylor, Losee & Fitzpatrick 2115* (T); Batchewana

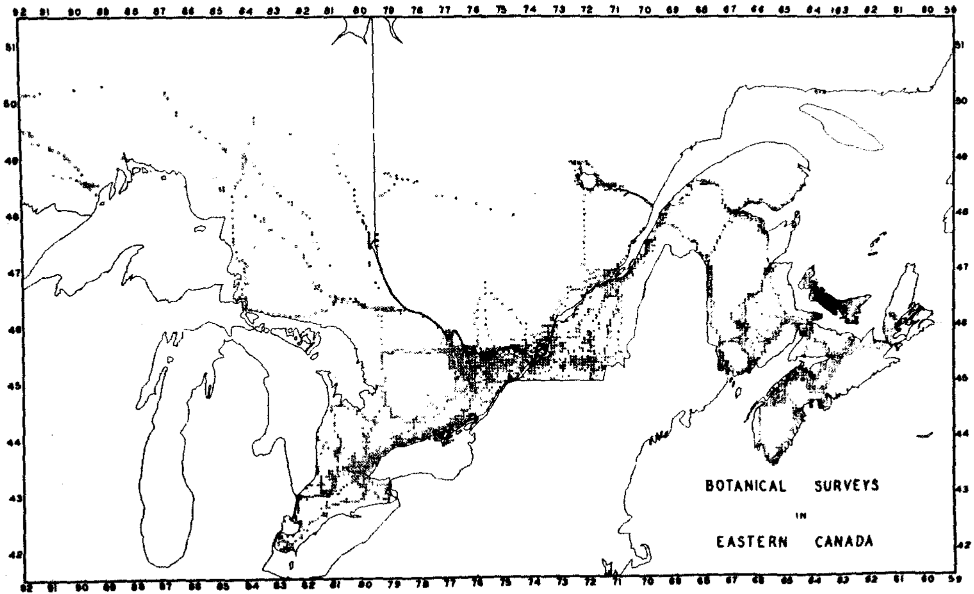


FIG. 6. *Points at which surveys have been made in the Canadian Weed Survey.*

River, *Taylor et al. 2116* (T); Michipicoten Harbour, *Hosie, Harrison & Hughes 170* (T).

A specimen in Nova Scotia Agricultural College Herbarium as follows is on the authority of Prof. A. E. Roland: Sandy river bank, Grand Falls, Victoria Co., N.B.

7b. *Prunus pumila* L. var. *susquehanae* (Willd.) Jaeg.

Ziergehoelze der Garten und Parkanlagen, ed. 1, 400. 1865; Rehder, Man. Cult. Trees and Shrubs, 466. 1927.

Prunus susquehanae Willd. Enum. Pl. Hort. Berol. 519. 1809; Fernald in Rhodora, 25: 73. 1923.

Prunus cuneata Raf. Ann. Nat. 11: 1820; Bailey, Cult. Nat. Plums and Cherries, 63. 1892; Robins. & Fern. in Gray's Man. ed. 7. 499. 1908; Britt. & Brown Illus. Fl. ed. 2, 2: 326. 1913; Wight, Nat. Am. Sp. Prunus, 67. 1915 (U.S. Dept. Agr. Bull. 179); Hedrick, Cherries of N.Y. 35. 1915;

Prunus pumila L. var. *cuneata* (Raf.) Bailey, Cycl. Am. Hort. 1451. 1901.

Specimens examined:

ONTARIO: Northumberland Co., *Macoun*, May 12, 1868 (McG), only foliage, and approaching, if not this variety; St. Williams, *Richardson*, 1919 (AA); Chatham, *McConnell*, June, 1876 (McG); Rondeau Park, *Faull*, June 29, 1905 (T); Sarnia, *Roy. Can. Inst.*,? May, 1925 (T); Bruce Peninsula, Big Andrew Lake, quaking margin, *Krotkov 10638* (G, T); Mud Lake, near Dyer's Bay, *Krotkov 10639* (G, T), Queenleen Lake bog, *Krotkov 10640* (G, T), Mud Lake, north of Emmett Lake, *Krotkov 10641* (T), Little Andrew Lake bog, *Krotkov 10642* (T), shrubby border of Moose Lake, *Krotkov 10643, 10644* (T), Conly Lake, gravelly shore, *Krotkov 10645* (G, T), Lymburner Lake shore, *Krotkov 10646* (T); North Bay, *Marie-Victorin, Rolland-Germain & Meilleur 45342* (M, T); Wanapitae, *Fernald & Pease 3394* (G, T); Espanola, *Fernald & Pease 3395* (G, US, Can, T).

Section 4. *Eucerasus* Koehne

8. *Prunus avium* L.

Fl. suec. ed. 2, 165. 1755; Robins. & Fern. in Gray's Man. ed. 7, 479. 1908; Britt. & Brown, Illus. Fl. ed. 2, 2: 327. 1913; Hedrick, Cherries of N.Y. 28. 1915 (see this for full synonymy); Rehder, Man. Cult. Trees and Shrubs, 471. 1927.

Common name: Mazzard, Sweet cherry.

Distribution: Europe and Western Asia; cultivated and escaped in Eastern Canada.

Specimens examined:

NOVA SCOTIA: Barton, *Jack 3181* (AA), naturalized; Wolfville, *Logan*, May 7, 1913 (A).

ONTARIO: Queenston Heights, *Macoun 34,448* (Can, T), *Scott*, May 24,

9. ***Prunus Cerasus*** L.

Sp. Pl. 474. *1753*; Robins. & Fern. in Gray's Man. ed. 7, 499. 1908; Britt. & Brown, Illus. Fl. ed. 2, 2: *327*. 1913; Hedrick, Cherries of N.Y. 24. 1915 (see this for full synonymy); Rehder, Man. Cult. Trees and Shrubs, 472. 1927.

Common name: Sour cherry.

Distribution: Western Asia to southeastern Europe; cultivated and escaped in Eastern Canada.

Specimens examined:

NOVA SCOTIA: Barton, *Jack 3182* (AA), naturalized.

PRINCE EDWARD ISLAND: Brackley Point Road, *Fernald, Long, & St. John 7677* (AA, Can, MV).

QUEBEC: Ste. Anne de la Pocatière, *Campagna 8035* (SteA).

ONTARIO: Niagara, *Scott*, May 24, 1897 (Can).

Section 5. *Mahaleb* Focke

10. *Prunus mahaleb* L.

Sp. Pl. 474. 1753; Bailey Cycl. Am. Hort. 1451. 1901; Robins. & Fern. in Gray's Man. ed. 7, 498. 1908; Britt. & Brown. Illus. Fl. ed. 2, 2: 328. 1913; Hedrick, Cherries of N.Y. 31. 1915 (see this for synonymy); Rehder, Man. Cult. Trees and Shrubs 473. 1927.

Common name: Mahaleb cherry.

Distribution: Europe and western Asia; cultivated as a stock for grafting and escaped in Eastern Canada.

Specimens examined:

ONTARIO: Queenston Heights, *Macoun*, May 18, 1901 (Can), *Scott*, May 17 and July 23, 1902 (T); Foster's flats, Niagara, *Macoun*, May 16, 1901 (T); Hamilton, *Dickson*, May 15, 1895, July, 1898 (Can).

11. *Prunus pennsylvanica* L. f.

Syst. ed. 31. Suppl. 252. 1781; Sargent, Silva N. Amer. 4: 35. pl. 156. 1892 (which see for full synonymy); *Robins. & Fern.* in Gray's Man. ed. 7, 498. 1908; *Britt. & Brown*, Illus. Fl. ed. 2, 2: 328. 1913; Wight, Nat. Am. Sp. *Prunus*, 60. 1915 (U.S. Dept. Agr. Bull. 179); Rehder, Man. Cult. Trees and Shrubs, 473. 1927; *Marie-Victorin*, Fl. Laurent. 370. 1935.

Common name: Bird cherry, Pin cherry, Wild red cherry.

Distribution: Canada and Northern United States. See Fig. 7.

Specimens examined:

NEWFOUNDLAND: St. John's, *Robinson & Schrenk*, Aug. 1, 1894 (G); Conception Bay, near Topsail, *Howe & Long 1333* (G); Conception Bay, Western Bay, *Torrey 104* (G); Avalon Pen., Whitebourne, Goose Pond, *Fernald, Long & Dunbar 26807;* Bay St. George, *Howe & Long 1051* (G); banks of Salmonier River, *Robinson & Schrenk 74* (AA, US, Can, McG); New Harbour, *Waghorne*, June 16, 1893 (AA, Q); Barred Islands to Fogo Island, *Sornborger*, Aug. 17, 1903 (G); Barachois Beach, *Kennedy 22* (G); Bay of Islands, Tweed Island, *Fernald, Long & Fogg 305* (G); Bay of Islands,

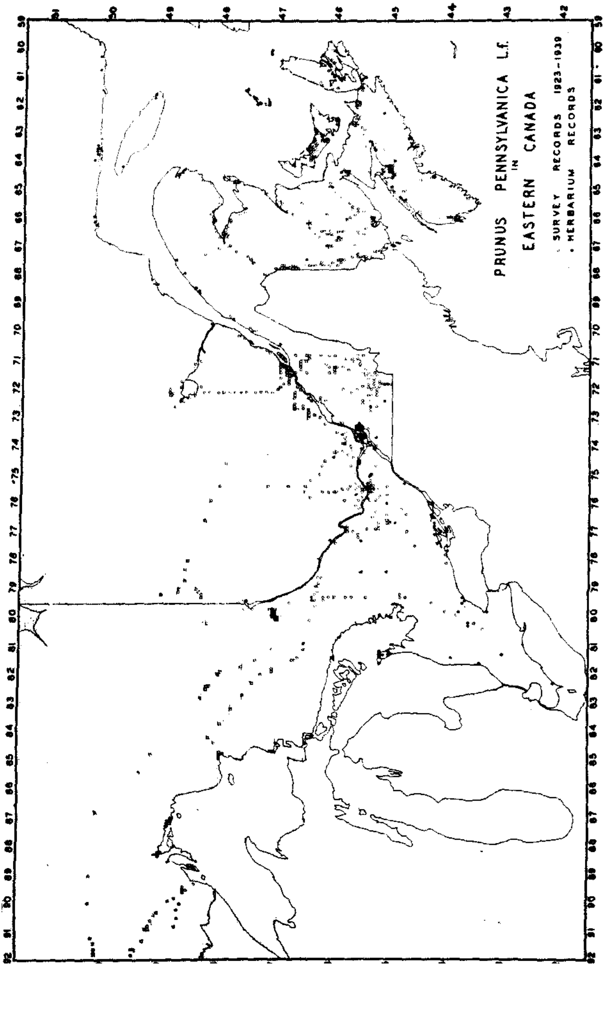


FIG. 7. Range of *Prunus pennsylvanica* L.f., Bird cherry.

Lark Harbour, *Waghorne 40* (G); Teddy Harbour, near Bonne Bay, *Bishop 395* (G); St. Pierre, chemin de l'Anne à Dinent, *Arsène 312* (G); Hamilton River, Labrador, *Low*, July 5, 1894 (Can); Mulligan River, Lake Melville, *Wetmore*, July 27, 1921 (Can).

Nova Scotia: St. Paul Island, between Lena Lake and Petrie's Pond, *Perry & Roscoe 263* (AA, G, Can); St. Paul Island, Top of Coggin Mt., *Perry & Roscoe 262* (G, Can); Ingonish, exposed slopes of Mt. Francy, *Nichols 1700* (G); Baddeck, *Macoun*, July 12, 1898 (Can); Canso, *Fowler*, July 26, 1901 (Q); Caledonia, *Faribault*, June 21, 1885 (M); Fraser's Mills, Antigonish, *Christie*, June 7, 1937 (A); Truro, *Burgess*, June 13, 1883 (T), *Jack 629* (AA); Halifax, *Macoun*, June 10, 1883 (Can), *Burgess*, June 20, 1883 (T); Mumford Road, Halifax, *Bell*, May 27, 1934 (Dal); Halifax Co., *King*, May 25, 1901 (Dal); Armdale, Halifax Co., *Facey*, June 14, 1934 (Dal); Armdale (Dutch Valley), *Fernald, Bartram & Long 24055* (G); Dartmouth, *Fernald, Bartram & Long 24054* (G); Newport, *Dill*, June 2, 1894 (AA); Five Mile River, Hants Co., *Pease & Long 21684* (G); Wolfville, *H. G. Perry*, May 15, 1911, May 21, 1911, May 21, 1912, *Wetmore*, June 9, 1920, *Mason*, May 26, 1919, *Rand*, Oct. 12, 1925 (A); near White Rock, *H. G. Perry*, May 22, 1913 (A); Kentville, *Fyles*, July 29, 1915 (DAO); Waterville, *Chute*, June 15, 1916 (A); Paradise, *Longley*, June 26, 1919 (A); Round Hill, *Jack 3195* (AA); Digby Co., Cedar Lake, *Jack 3765* (AA, G); Central Port Mouton, *Fernald, Bissell, Graves, Long, & Linder 21685* (AA, G); Yarmouth, *Macoun*, May 23, 1910 (Can); *Pease & Long 21683* (G); Brazil Lake, Yarmouth Co., *Bartram & Long 24053* (G); Pleasant Valley, Yarmouth Co., *Jack 3153* (AA).

Prince Edward Island: Mt. Stewart, *Fernald, Bartram, Long & St. John 7676* (AA, G, US, Can, MV); Brackley Point, *Macoun*, June 22, 1888 (Can).

New Brunswick: Sackville, *Goodwin*, June, 1893 (Q); Moncton, *L. M. Perry*, May 28, 1920 (A); Rothesay, *Matthews*, July 6, 1879 (T); Lily Lake (near St. John), *Hay*, June 9, 1876 (NBM); Campbell's Island, *Smith*, July 17, Aug. 20, 1888 (US); Fredericton, *Jack 586* (AA); Fielding, *E. Richardson*, May 25, 1923 (A); Campbellton, *Chalmers*, June 13, 1876 (Can).

Quebec: Magdalen Islands, Amherst Island, *St. John 1902* (G); Magdalen Islands, Grindstone Island, *Fernald, Bartram, Long & St. John 7675* (G); Iles de la Madeleine, "La Montagne", Ile du Havre au Ber, *Marie-Victorin & Rolland-Germain 9511* (US, M, MV); Magdalen Islands, Ile Brion, *Marie-Victorin & Rolland-Germain 9510, 9749* (M, MV); Anticosti, Rivière du Renard, *Marie-Victorin & Rolland-Germain 27246* (M), *27384* (AA, Can, M, MV); Anticosti, Potato River, *Adams*, Aug. 3, 1936 (DAO); Sept-Iles, La Grosse-Boule, *Marie-Victorin & Rolland-Germain 18701* (M, MV); Pointe-aux-Esquimaux, *Marie-Victorin & Rolland-Germain 18702* (M, MV, US); Eskimo Point, *Townsend*, June, 1909 (G); Mingan, Seign., *St. John*, Sept. 18, 1895 (Can); Matamek River district, *Bowman 49, 94,*

236 (G); Manowin Island, Seven Islands, Saguenay Co., *Lewis*, Sept. 12, 1928 (Can); Betchewan, Saguenay Co., *Lewis*, Aug. 26, 1928 (Can), *Abbe 1142* (G); Gaspé Bay, *Jack*, Aug. 23, 1927 (AA); Gaspé Basin, *Fowler*, Aug. 16, 1905 (Q); Mont Saint Pierre, Gaspé Co., *Marie-Victorin, Rolland-Germain* & *Jacques 33198* (AA, M, MV); Maguasha, *Fowler*, July 5, 1905 (Q); Carleton, *Adams*, Aug. 18, 1935 (DAO); Matane near the Matane River, *Forbes 2022* (G); Little Metis, *Fowler*, Aug. 17, 1906 (G, Q); Rimouski, *Lemay*, 1914 (SteA); Bic, *J. Rousseau 26331, 30006* (M, MV); Ilets Rousseau (Temis.), *Campagna, Caron* & *Rousseau*, Aug. 18, 1927 (SteA); Tadoussac, *Kennedy*, Aug., 1892 (G); Ste. Anne de la Pocatière (Kam.), *Campagna*, May, 1931 (SteA); St. Felicien, Shanusham Falls, *Kennedy*, July 18, 1892 (G); Montmagny, *J. Rousseau 20891* (M); St. Famille de l'Ile d'Orleans, *Rousseau 24664, 25247* (M); Montmorency Falls, *Macoun*, July 1, 1905 (Can); Charlesbourg, *Michel*, June 1, 1933 (M); Black Lake, Cariboo Hill, Megantic Co., *Fernald* & *Jackson 12116* (G); Thetford, *Marie-Victorin 11191* (MV, M); vicinity of Trois Rivières, *Stanislas 449* (M); Ste. Maurice River, 10 mi. above Grandes Piles, *Jack*, Sept. 24, 1896 (AA); St. Louis de France, Champlain Co., *Adrien 1634* (AA, M); Lake Knowlton, near Brome, *Knowlton*, May 27, 1923 (G); North Hatley, *Jack*, Sept. 26, 1914 (AA); Farnham, *Jack*, June 2, 1914 (AA); Chateauguay Co., *Jack 3931* (AA); Montreal, Mont Royal, *Marie-Victorin* & *Rolland-Germain 34181* (M, AA, MV), *Majoric 20885* (M), *Mignault*, May 4, 1878 (M); Mont Ste. Anne, environs Montreal, *Marie-Victorin 20890* (M); Longueuil, *Marie-Victorin 4329* (MV, AA, US), *188* (US, MV), *8295* (MV), *11189* (M), *Rolland-Germain 29419* (M, MV); Nichols, Montreal, *Holmes*, May 22, 1821 (McG); La Trappe, *Louis-Marie 24206* (M); L'Epiphanie, *Louis-Marie*, Aug. 5–7, 1930 (Can); St. Jerome, *Marie-Victorin 668* (MV); St. Jovite, Lac Ouimet, *Churchill* (G); Farm Point, *Macoun 87174* (Can); Blueberry Point, Aylmer, *Macoun 59915* (Can); north end of Durant Lake, Atik River, *Wilson*, Sept., 1907 (Can); Eastmain, east coast James Bay, *Potter 488* (G), open woods.

ONTARIO: 5½ mi. southeast of Hawkesbury, near Little Rideau Creek, *Minshall 1500*, fairly common (DAO); 3 mi. east of Hawkesbury, *Minshall 1563* (DAO); Ottawa, *Ami*, May, 1879 (McG), *Fletcher*, May 21, 1879 (McG), Billings Bridge, *Fletcher*, May 15, 1878 (DAO), *Harrington*, May 21, 1906 (Can), Rockcliffe, *Scott*, May 28, 1892 (T), *Macoun*, May 19, 1911 (Can), *E. G. Anderson*, May 19, 1932 (OAC); Prescott, *Billings*, May 20, 1862 (OAC); Jones Falls, *Fowler*, May 20, 1891 (Q); Killaloe, *Milton Jack*, 1903 (AA); Newburgh, *Fowler*, May 14, 1897 (US); Plevna, *Fowler*, Aug. 5, 1902 (G, Q); Hastings Co., *Macoun*, May 12, 1870 (McG); Kingston, *Fowler*, July 23, 1900 (Q), May 23, 1884 (US); Redstone Lane, Haliburton Co., *Krotkov*, Sept. 19, 1929 (T); Humber Plains, *Scott*, May 9, 1896 (Can); Belleville, *Macoun*, May 24, 1871 (Can); Toronto, *Turner*, June 3, 1930 (T); vicinity of Edmonton, *White*, May 17, 1890 (McM); Snelgrove, *White*, May 21, 1898 (Can); Guelph, *Simmons* & *Van Loon*, May 18, 1936 (OAC),

Hart, May 22, 1930 (OAC); Hamilton, *Burgess*, May 21 and Aug. 17, 1889 (McM); Lily Pond Mills, London, *Millman*, May 23, 1884 (T); Aylmer, *R. T. Anderson*, May 23, 1898 (T); Aylmer, *R. T. Anderson*, May 23, 1898 (T); Wingham, *Morton*, June 1890 (Can, OAC); Johnston Harbour, Lake Huron, *Macoun*, Aug. 22, 1901 (Can); Georgian Bay Biological Station, no other data (Can); Port Cockburn, Muskoka, *Burgess*, Aug. 26, 1882 (T, McG); Bruce Peninsula, Stokes Bay, *Krotkov 9146* (US, T, G), *9147* (US, T), Lion's Head, *Watson*, Aug. 7, 1926 (T), Emmett Lake, *Krotkov 10649* (T), Dyer's Bay, *Krotkov 10648* (T), Cabot Head, *Krotkov 9842, 9843* (T), Tobermory, *Krotkov 7532* (T); Muskoka, *R. W. Smith*, no other data (McM); Chalk River, Ottawa Valley, *Macoun*, May 30, 1884 (Can); Cache Lake, Algonquin Park, *Macoun*, May 30 and July 17, 1900 (Can); North Bay, *Miller*, Aug. 21, 1896 (G), *Ricker*, June 4, 1926 (T); clay belt region, *Tunstell*, 1910 (T); Bear Island, Timagami F. R., *Krotkov 5406, 5407* (T), *5745* (G, T); Devil's Lake, Timagami F. R., *Krotkov 5408* (T); Timagami Forest Reserve, *Watson*, June 23, 1922 (Can); Timagami Forest Reserve, *Watson 712?, 5752, 6841, 6842* (T); Twin Islands, Timagami region, *E. & D. M. Anderson 26041* (G); Mamainse Mt., Algoma district, *Taylor, Losee & Fitzpatrick 2110* (T); Batchewana Bay, *Taylor, Losee & Fitzpatrick 2111* (T); Mamainse Pt., *Taylor, Losee & Fitzpatrick 2112* (T); Sawyer Bay, *Taylor, Losee & Bannan 1251, 1252* (T); Shangoina Island, *Taylor, Losee & Bannan 1253, 1254* (T); Killarney, *Scott*, May, 1895 (T); Nipigon, Lake Superior, *Macoun*, June 23, 1884 (Can); Michipicoten Harbour, *Hosie, Harrison & Hughes 165, 166, 167* (T); Schreiber, *Hosie, Losee & Bannan 286, 288, 289, 290* (T).

Specimens in Nova Scotia Agricultural College Herbarium as follows are on the authority of Prof. A. E. Roland: Mixed woods on the ridge above Wolfville; Blomidon; both Kings Co., N.S.

Records from Canadian Weed Survey: These together with the above citations are to be seen in Fig. 7.

SUBGENUS IV. *Padus* Koehne

12. *Prunus serotina* Ehrh.

Beitr. 3: 20. 1788; Sargent, Silva N. Amer. 4: 45. pl. 159. 1892 (which see for full synonymy); Robins. & Fern. in Gray's Man. ed. 7, 497. 1908; Fernald in Rhodora, 18: 140. 1916; Rehder, Man. Cult. Trees and Shrubs, 474. 1927; Marie-Victorin, Fl. Laurent. 321. 1935.

Padus virginiana (L.) Mill. Gard. Dict. ed. 8. No. 3. 1768; Britt. & Brown, Ill. Fl. ed. 2, 2: 329. 1913.

Common name: Black cherry, Rum cherry.

Distribution: Eastern Canada and United States. See Fig. 8.

Specimens examined:

NOVA SCOTIA: St. Croix, *Jack 3324* (AA, G), *3693* (AA); Newport, *Jack 728* (AA); Kentville, Agricultural Experiment Station, *McLellan*, June 20, 1939 (DAO); west of Bridgewater, *Fernald & Long 24051* (G);

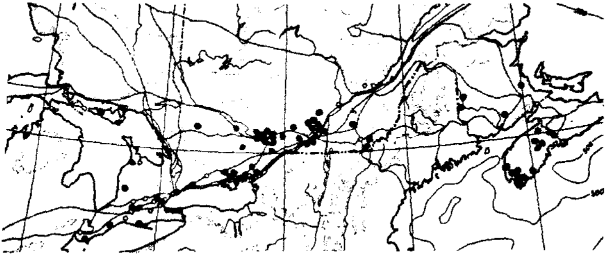


FIG. 8. *Range of Prunus serotina Ehrh., Black cherry.*

New Germany, *Hamilton*, June, 1891 (Can); Bridgewater, *Macoun*, July 15, 1910 (Can); Meteghan, *Fernald & Long 21676* (AA, G); Clyde River, *Jack 3449* (AA, G); Belleville, *Long & Linder 21677* (G); Tusket Falls, Tusket River, *Fernald, Bissell, Graves, Long & Linder 21678* (G, Can); *Argyle, Jack 3428* (AA); Pleasant Valley, *Jack 3135* (AA).

NEW BRUNSWICK: Bass River, *Fowler*, 1874 (Q); Norton, *Hay*, June 11, 1878 (NBM); Florenceville road to Centreville, *H. G. Perry*, June 23–30, 1910 (A); Tracey Mills, Carleton Co., *H. G. Perry*, July 13, 1910, July 21, 1910 (A).

QUEBEC: Danville, *Marie-Victorin, Rolland-Germain & Meilleur 43325* (M), seedlings from border of woods unsupported by typically mature specimens; St. Paul, Arthabaska Co., *Campagna*, July 20, 1922 (SteA), vegetative shoot only; environs Longueuil, *Marie-Victorin 9509* (M), *Marie-Victorin & Rolland-Germain 46755* (T); Ste. Clothilde, *Jack*, May 30, 1904 (AA); Chateauguay, *Marie-Victorin 28325* (M, MV); Montreal, *Lyman*, June, 1879 (McG); Bois des Filion, *Marie-Victorin 28316* (AA, M, MV); St. Janvier, *Jack*, June 2, 1904 (AA); St. Timothée, *Jack*, Sept. 19, 1906 (AA); St. Jerome, *Mignault*, June 1, 1878 (M); vicinity St. Jerome (Laurentides), *Marie-Victorin 3165* (MV); Rawdon, *Marie-Jean-Eudes 702* (M); Hull, *Macoun*, June 1, 1903 (DAO, Can); near Hull cemetery, *Macoun*, Aug. 29, 1874 (Can); Chelsea, *Fletcher*, no date (DAO), *Harrington*, Sept. 11, 1909 (Can); King Mountain (near Hull), *Marie-Victorin 15537* (M, MV); Deschenes, *Rolland 10196* (M, MV); Lake Deschenes on Ottawa River, *Rolland, 15536* (M, MV); Kirk's Ferry, *Harrington*, May 27, 1906 (Can).

ONTARIO: 2 mi. west of Vankleek Hill, *Minshall 1418* (DAO); Ottawa, *Macoun*, Aug. 30, 1911 (Can); *Scott*, June 11, 1931 (Can); May 31, 1892, June 1, 1892 (T), *E. G. Anderson*, June 6, 1933 (DAO); Ottawa West, *Minshall*, May 28, 1935, *Groh*, June 7, 1935 (DAO); Spencerville, *Minshall 873* (DAO); Navan, *Macoun*, Sept. 4, 1911 (Can); Almonte, Forsythe Farm, *Minshall 1747* (DAO); Renfrew Co., Bromley Twp., Con. V, 4 mi. north-

east of Douglas, *Minshall 1750* (DAO); Kingston, *Fowler*, Sept. 8, 1900 (Q); Gananoque, *Fowler*, Aug. 18, 1887 (Q); Collin's Bay, *Fowler*, June 11, 1902 (G, Q); Belleville, *Macoun*, June 10, 1878 (Can); Hastings Co., Sidney Twp., 1½ mi. northwest of Chatterton, *Minshall 602* (DAO); Hastings Co., *Macoun*, June 24, 1871, May 7, 1873 (McG); Bayside, (Hastings Co. ?) *Faull* (T); Battersea, *Fowler*, May 28, 1904 (Q); Hampton, *Allin*, June 18, 1927 (T); Toronto, *Scott*, May 20, 1896 (Can); Guelph, *Dept. of Botany*, June 7, 1929, *Minshall*, June 7, 1932 (OAC); Hamilton, *Senn*, June, 1932 (McM); Lincoln Co., *McCalla*, June 5, Sept. 24, 1897 (Can); 2 mi. west of Niagara, *Dearness*, Aug., 1891 (Can); Foster's Flats, Niagara, *Scott*, May 24, 1898 (Can); St. Williams, *Richardson*, Sept. 5, 1919 (AA); London, *Millman*, May 27, 1881 (T), *Burgess*, May 28, 1878, Aug. 20, 1881 (McG); Aylmer, *R. T. Anderson*, May 24, 1899 (T); Leamington, *Macoun*, May 25, 1901 (Can); Wingham, *Morton*, June 3, 1891 (Can); Red Bay, Bruce Peninsula, *Watson*, Aug. 29, 1926 (T); Cache Lake, Algonquin Park, *Macoun*, June 7, 1900 (Can).

A specimen in Nova Scotia Agricultural College Herbarium as follows is on the authority of Prof. A. E. Roland: Thicket, Clyde River, Shelburne Co., N.S.

13. *Prunus Padus* L.

Sp. Pl. 473. 1753; Rehder, Man. Cult. Trees and Shrubs, 475. 1927 (which see for synonymy).

Common name: European bird cherry.

Distribution: Europe and northern Asia; rarely naturalized in Eastern Canada.

Specimens examined:

ONTARIO: Ottawa, Rideau Canal, *Minshall*, May 15, 1934 (DAO), Dow's Swamp, *Minshall*, May 22, 1935 (DAO); doubtless escaped in both cases from the Dominion Arboretum plantings.

14. *Prunus virginiana* L.

Sp. Pl. 473. 1753; Sargent, Silva N. Amer. 4: 41, pl. 158. 1892 (which see for full synonymy); Robins. & Fern. in Gray's Man. ed. 7,498. 1908; Fernald in Rhodora, 18: 140. 1916; Rehder, Man. Cult. Trees and Shrubs, 476. 1927; Marie-Victorin Fl. Laurent. 322. 1935.

Padus nana (Du Roi) Roemer, Arch. 1 (2): 38. 1797; Britt. & Brown, Illus. Fl. ed. 2, 2: 329. 1913.

Common name: Choke cherry.

Distribution: Eastern and Central Canada and North-Central and Eastern United States. See Fig. 9.

Specimens examined:

NEWFOUNDLAND: Gravelly beach of Rushy Pond, valley of Exploits River, *Fernald* & *Wiegand* assisted by *H. T. Darlington*, Aug. 28, 1911 (Can); Laurentian area at head of Exploits River system, Little Red Indian Lake, *Fernald* & *Wiegand 5732* (AA); woods along the Salmonier River, *Robinson* &

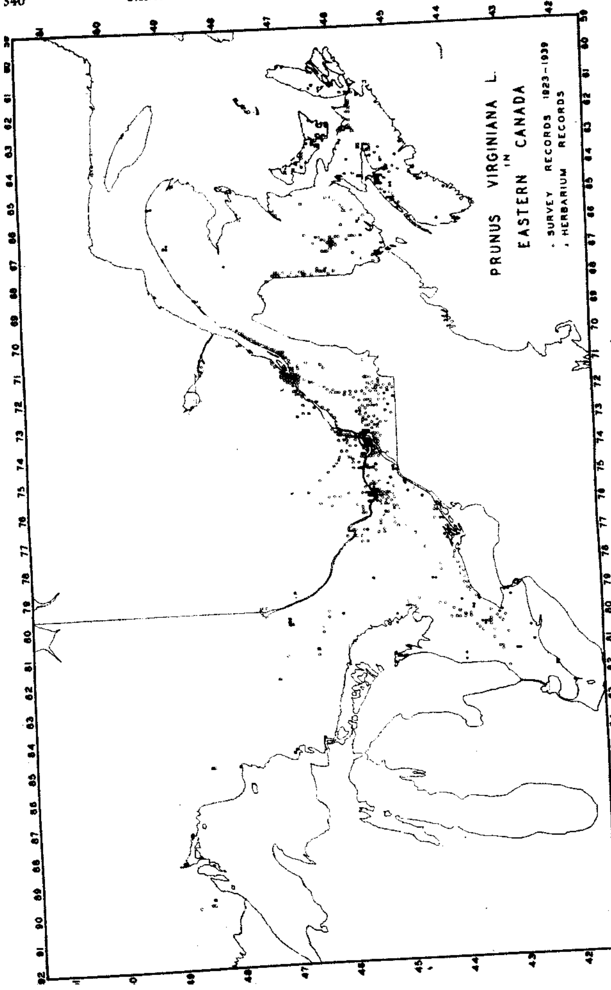


FIG. 9. *Range of Prunus virginiana L., Choke cherry.*

Schrenk 75 (AA, Can, McG); Bay of Islands, Birchy Cove, *Waghorne*, June 20, 1895 (AA, Q); Bay of Islands, Mt. Musgrave and Humber mouth, *Fernald & Wiegand 3626* (G).

NOVA SCOTIA: Valley of Barachois River, *Nichols 1363* (G); Big Intervale, Margaree, *Macoun*, July 18, 1898 (Can); Port Bevis, *Fernald & Long 21682* (G), open woods about base of gypsum cliff; Fraser's Mills, Antigonish, *Christie*, June 16, 1935 (A); Boyleston, *Hamilton*, June 1, 1890 (Can); Pictou, *Jack 783* (AA); Truro, *Jack 633* (AA); Armdale, Halifax Co., *Bell*, June 14, 1934, *Facey*, June 14, 1934 (Dal); Calkin's Farm, Windsor Road, Halifax Co., *Lawson*, June 26, 1891 (AA); Windsor, near Wentworth gypsum quarries, *Fernald, Bartram & Long 24052* (G); Newport, *Dill*, June 2, 1894 (AA); Five-mile River, Hants Co., *Pease & Long 21680* (G); West Gore, Hants Co., *Wallace*, June 15, 1932 (A); Black River, King's Co., *Wetmore*, June 12, 1918 (A); Paradise, *Longley*, June 10, 1919 (A); Black River Falls, *J. H. Perry*, June 3, 1932 (A); Wolfville, *King*, June 8, 1901 (Dal), *H. G. Perry*, June 5, 1912, June 18, 1913, *Mason*, June 10, 1919 (A); Kentville, *McLellan*, June 6, 1939 (DAO); Waterville, *Chute*, June, 1915 (A); Paradise, *Longley*, June 10, 1919 (A); Bridgewater, *Jack 3512* (G, AA), *Macoun*, July 25, 1910 (Can); Port Mouton, *Bissell & Graves 21681* (G, Can); roadside near Digby, *Howe & Long 285* (G); Corberrie, *Jack 3123* (AA); Tusket, *Long & Linder 21679* (AA, G).

PRINCE EDWARD ISLAND: Charlottetown, *Fernald, Long & St. John 7674* (G); Brackley Point, *Macoun*, Aug. 31, 1888 (Can); Malpeque, *Fowler*, July 14, 1904 (Q).

NEW BRUNSWICK: St. Andrews, *Fowler*, June 18, July 10, 1900 (Q); Fredericton, *Fowler*, Aug. 4, 1880 (Q), *Sargent*, Aug. 2, 1912 (AA); Woodstock, *Fernald & Long 13940* (G); Bathurst, *Meehan*, May, 1880 (McG); Restigouche River, *Chalmers*, June 17, 1876 (Can); Tracey Mills, *H. G. Perry*, July 13, 1910 (A); Hampton, *Hay*, June 10, 1876 (NBM); Emerson, *Fowler*, July 14, 1887 (Q).

QUEBEC: Grosse Island, Magdalen Ids., *Fernald, Long & St. John 7673* (G); Brion Island, Magdalen Ids., *St. John 1906* (G); Rivière Vaureal, Anticosti, *Marie-Victorin, Rolland-Germain & Louis-Marie 20889* (M, MV), *Marie-Victorin & Rolland-Germain, 27370* (Can, M, MV); Douglastown, *Collins, Fernald & Pease 6407* (G); between Lac Mont Louis and north fork of Madeleine River, Gaspé Co., *Fernald, Dodge & Smith 25869* (G, Can) Nettle Gully, alt. 400 m., northern base of Mt. Collins, *Fernald, Griscom, Pease & Smith 25867* (G, Can, MV); northern base of Mt. Mattaouisse, *Fernald, MacKenzie & Smith 25868* (G, Can, MV); woods at about 500 m. alt. in Fernald Basin between Mts. Mattaouisse and Fortin, *Fernald & Smith 25870* (G, Can, MV); Metis Park? *Baylis?*, May 30, 1885 (T), specimen with bifurcated inflorescence; Bic, *Forbes*, June 25, 1905 (Can); Cap Enrage (Rimouski Co.), *J. Rousseau 26488* (MV); Ste. Anne (Kam.), *Campagna*, June 1931 (SteA); Chicoutimi, *Marie-Anselme*, June 15, 1933 (M); Grand'Mere, *Adrien 1665* (MV); Sainte-Marie, Champlain Co., *Stanislas*

447 (M); Montmorency Falls, *Macoun*, July 3, 1905 (Can); Berthier en Bas, *J. Rousseau 26236* (Can, M, MV)), *Marc Rousseau 30066* (M, MV); Wolfe's Cove below Quebec, *Sargent*, Aug. 4, 1912 (AA); Plains of Abraham (Quebec), *Romeo 1766* (M); Levis, *Jack*, June 1, 1903 (AA); Phillipsburg, *Knowlton*, May 24, 1925 (G); Massawippi, *Jack*, Sept. 26, 1914 (AA); Stanstead, *Jack*, June 2, 1914 (AA); St. Dominique, *Marie-Victorin* & *Rolland-Germain 34041* (M), *34042* (M, MV); Longueuil, *Marie-Victorin*, *9475*, *11190* (M), *Rolland-Germain 29433* (M), *Marie-Victorin 277*, *278*, *667* (MV); Montreal, *Lyman*, May, 1879 (McG), *Mignault*, May 10, 1878 (M); Nichol's (Montreal), *Holmes*, May 29, 1821 (McG); St. Laurent, *Adrien 999* (M); Mont Royal, *Marie-Victorin 21735* (M, MV); Ile Bizard, *Adrien 1700* (M, MV) *1701* (MV); Ste. Anne, Montreal Isd., *Jack*, May 29, 1913 (AA); Highlands, *Jack*, May 21, 1900 (AA); Verdun, *Jack*, May 30, 1902 (AA); La Trappe, *Louis-Marie 2482* (M); Grenville, *Mignault*, May 1, 1878 (M); L'Epiphanie, *Louis-Marie*, *Dudemaine* & *Laporte 1548* (Can); Ste. Agathe des Monts, *Jack*, May 29, 1903 (AA); Rivière Ouareau, Rawdon, Montcalm Co., *Marie-Jean-Eudes 556* (M); Hull, *Harrington*, May 26, 1906 (Can); Aylmer, *Harrington*, June 3, 1905 (Can); environs of Ottawa, *Marie-Victorin 9129* (M); Fort Temiscamingue, *Marie-Victorin*, *Rolland-Germain*, & *Meilleur 43948* (M).

ONTARIO: 5½ mi. southeast of Hawkesbury, near Little Rideau Creek, *Minshall 1501* (DAO); 1 mi. west of Vankleek Hill, *Minshall 1383*, roadside fencerow common (DAO); Kirkhill, *Minshall 1295* (DAO); Lochiel, *Minshall 1340*, common (DAO); 6 mi. northeast of Cornwall, Leitch farm, *Minshall 1241* (DAO); Cornwall, Flanagan's Point, *Jack*, May 29, 1914 (AA); 2½ mi. southeast of Finch, Eligh farm, *Minshall 1176* (DAO); 2 mi. east of Iroquois, *Minshall 1213*, *1214* (DAO); Navan, *Minshall 1613* (DAO); Spencerville, *Minshall 867* (DAO); Ottawa, Rockcliffe Park, *Macoun*, May 21, 1903 (Can, DAO), May 19, 1911 (Can), Billings Bridge, *Fletcher* (DAO), Canal Road, *Minshall*, May 22, 1937, May 28, 1935, May 27, 1937 (DAO), Dow's Swamp, *Harrington*, May 30, 1906 (Can); Ottawa West, *Minshall*, May 27, 1937 (DAO); west of Ottawa, *Macoun 87176* (Can); Lyn, *Minshall 839* (DAO); Almonte, Forsythe farm, *Minshall 1642*, common along fencerows (DAO); Carleton Place, *Macoun*, May 31, 1884 (Can); Renfrew Co., Bromley Twp., Con. V, 4 mi. northeast of Douglas, *Minshall 1751*, roadside fencerows, common (DAO); Gananoque, *Fowler*, Aug. 24, 1884 (Q); Kingston, *Fowler*, June 2, 1897 (Q); Kingston Mills, *Ford*, May 23, 1894 (Q); Plevna, *Fowler*, Aug. 18, 1902 (G); Picton, *Fowler*, June 3, 1902 (Q); Belleville, *Macoun*, June 15, 1870 (Can), June 1878 (OAC); Lindsay, *Scott*, Aug. 16, 1903 (T); Cameron Lake, Victoria Co., *Krotkov*, Sept. 10, 1929 (T); Toronto, Bathurst St., near Eglinton, *R. W. Smith*, June 2,? (McM); North Toronto, collector not stated, May, 1894 (McM); Edmonton, *White*, May 17, 1890 (McM), May 25, 1891, July 31, 1893 (Can); Aurora, collector not stated, nor date (McM); Cressy, *Oughton*, June 10, 1928 (T); Cartwright, *Scott*, Aug. 11, 1898 (T); Guelph,

Dewar, (OAC), *Hamilton* (T), both without dates, *Doherty*, July 10, 1896 (OAC); Hamilton, *Clappison*, June 21, 1898 (T), May 25, 1900 (T); De Cew Falls, Lincoln Co., *McCalla*, May 22, 1897, July 26, 1897 (Can); Niagara, *Potter*, 1908 (T); Foster's Flats, Niagara River, *Scott*, May 24, 1898 (T); St. Williams, *Richardson*, 1919 (AA); London, *Millman*, May 23, 1881, Aug. 10, 1881 (T), *Burgess*, Aug. 10, 1881 (McG); Aylmer, collector not stated, May 27, 1898 (T); Rondeau Park, *Faull*, May 21, 1915, June 5, 1915 (T); Pt. Pelee, *Macoun*, Aug. 6, 1901 (Can); Wingham, *Morton*, May 28, 1891 (Can); Huron Co., *Fraser*, June 7, 1928 (T); Cape Croker, *Watson 3408* (T); Bruce Peninsula, Umbrella Lake, *Krotkov 9150* (T), Stokes Bay, *Krotkov 9149* (T), Dyer's Bay, *Krotkov 10647* (T); Red Bay, *Watson 3602* (T); Cabot Head, *Krotkov 9841* (T); Devil's Island, Tobermory, *Krotkov 7535* (G, T); Georgian Bay, *Huntsman?*, July 18, 1905 (Can); Cache Lake, Algonquin Park, *Macoun*, June 7, Aug. 11, 1900 (Can); Frank's Bay, Lake Nipissing, *Fallis*, June 11, 1929 (T); Timagami Forest Reserve, *Watson 6843* (G, T) *6844* (T), *Krotkov 5409* (T); Bear Island, Timagami Forest Reserve, *Krotkov 5410* (G, T); Garden River, Algoma Co., *Fassett 14736* (G); Batchewana Village, *Taylor*, *Hosie*, *Fitzpatrick*, *Losee & Leslie 2114* (T); Mamainse Mtn., *Taylor*, *Hosie*, *Fitzpatrick*, *Losee & Leslie 2113* (T); Sawbill Lake, *Taylor*, *Losee & Bannan 1255* (T); Nipigon, Lake Superior, *Macoun*, June 26, 1884 (Can); Michipicoten Harbour, *Hosie*, *Harrison & Hughes 168*, *169* (T); Schreiber, *Hosie*, *Losee & Bannan 292*, *293* (T).

Specimens in Nova Scotia Agricultural College Herbarium as follows are on the authority of Prof. A. E. Roland: Sandy hillsides, common, south branch of the North R., Colchester Co.; Blomidon, roadside, Kings Co.; roadside, Antigonish; Lornedale near Truro, Bible Hill, Truro, and swamp, Truro, all N.S.

Records from Canadian Weed Survey: together with the above citations are to be seen on Fig. 9.

Analysis of Choke Cherry *(P. virginiana)* and Bird Cherry *(P. pennsylvanica)* Records

Incidence of both choke cherry and bird cherry has been further derived by charting the records taken from the Canadian Weed Survey, the percentage incidence* being charted for each meridian. The 2805 surveys in Eastern Canada are distributed somewhat in keeping with uneven settlement, but their unevenness apparently does not vitiate the results.

From a study of the chart (Fig. 10) in conjunction with the maps the following seem significant deductions:

North and west of Georgian Bay, from rather sparse surveys, *P. virginiana* is almost absent, while *P. pennsylvanica* becomes increasingly and extra-

* *The term percentage incidence is used for the numerical value obtained by rendering the number of records for a given species within one meridian into a percentage of the surveys within that meridian.*

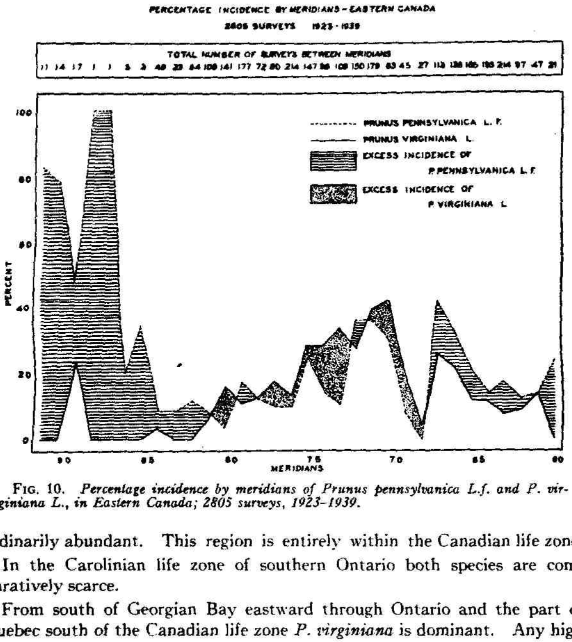


FIG. 10. *Percentage incidence by meridians of Prunus pennsylvanica L.f. and P. virginiana L., in Eastern Canada; 2805 surveys, 1923–1939.*

ordinarily abundant. This region is entirely within the Canadian life zone.

In the Carolinian life zone of southern Ontario both species are comparatively scarce.

From south of Georgian Bay eastward through Ontario and the part of Quebec south of the Canadian life zone *P. virginiana* is dominant. Any high incidence of *P. pennsylvanica* within these meridians is mostly from surveys in the Laurentians to the north, as for instance along the lines from Quebec city to Lake St. John district, and from North Bay to the Abitibi district. The same influence is evident in a small way at the point where the northern belt reaches through to the St. Lawrence at Kingston.

From Meridian 68° to 69° surveys are few, and records of both species are few in number and in percentage incidence. The same situation prevails at a point north of Lake Superior and an increased number of surveys in both cases would be desirable.

From the western boundary of New Brunswick to the Atlantic *P. pennsylvanica* is almost entirely in the ascendency, particularly so where the broken land of eastern Cape Breton, and the prevailingly light soils of Prince Edward Island and of Victoria and Carleton counties in New Brunswick exert their influence. These points are likewise in the Canadian life zone.

Discussion and Conclusions

Prunus species and varieties to the number of sixteen are recognized from within the eastern half of Canada. These include five plums and the peach, three sand cherries, regarded as varieties of one species, and six cherries. Two plums, the sand cherries, and three other cherries are native, the others all escapes from cultivation.

The relative abundance in the wild state of the various species and varieties is fairly well reflected in the volume of citations brought together. Distribution not being the same for each, abundance locally for some may be greater, however, than the total of citations would suggest. Introduced species, likewise, are too sparingly collected to indicate adequately their complete distribution. It is a safe assumption that nowhere do they constitute a large part of the wild *Prunus* population. Planted plums and cherries, subject to the usual orchard protection practices, are not likely either to be major sources of trouble through harbouring insect vectors of disease.

Of the two native plums, *P. americana* is virtually confined to the south-western peninsula of Ontario, where it is evidently sufficiently abundant in the peach-growing district to be of importance in relation to disease transmittal. *P. nigra* occurs in the Maritimes, Quebec, and Ontario, but in southern Ontario it appears to be near its southern limits, overlapping the northern limits of *P. americana*, and it is doubtful whether it shares much responsibility with the other for any trouble caused the fruit grower. To potato growers in parts of Eastern Canada it may be found to be of importance.

The sand cherries (*Prunus pumila*), taken together, are quite widely distributed in the area, but of all the *Prunus* are least in proximity to agricultural settlements. Two are largely confined to Ontario shores and wild land; the other, variety *depressa*, occurs from Ontario to the Atlantic. The more upland habitat ascribed by some writers to the variety *susquehanae* as contrasted with the shore-loving *pumila*, may have some confirmation from these Canadian sources, but overlapping along the Great Lakes system is considerable.

Of the three remaining wild cherries, the black cherry has a range well within the northern limits of bird and choke cherry. It occurs only in the more southerly half of Nova Scotia, sparingly in New Brunswick (where some treatments show it absent), and in southern Quebec and Ontario below a line following the St. Lawrence and Ottawa valleys and across to the north of Lake Huron. We have seen no specimens to substantiate reported occurrences west of Lake Superior. Within its range black cherry is to be found in deep soils in woodlands and along fence rows.

Choke cherry and bird cherry have a wide distribution throughout our area and farther west. They are often found growing together, but in a general way it may be said that choke cherry frequents the fencerows and environs of farm land, while bird cherry occupies sandier tracts and broken land and all the wilderness to the north. This difference is clearly revealed in the mapping of the two species at such points as the Laurentian country

between Quebec city and Lake St. John, and the sandy tracts beyond the lake, and along the St. Lawrence river below Quebec, where bird cherry predominates on the rocky north shore and choke cherry is more plentiful in settlements along the opposite side. The rather sparse surveys right across from Lake St. John to Lake Superior are nevertheless numerous enough to show dissimilar incidence of the two species.

A study of the maps shows a few areas where neither species is prominent. At least some of these are similar in their lowland plains aspect, i.e., the Valleyfield-Huntingdon area south of Montreal and the southern tip of Ontario.

These and similar but less obvious places would seem to be the ones to be further surveyed and studied in the search for a region for the production of disease-free foundation stock. The Bay of Chaleur potato-growing district is obviously better situated than the Victoria-Carleton district of the same province. The lower St. Lawrence and the Shipshaw area near Chicoutimi, both of which are being considered as to suitability, appear only moderately safe, but surveys are scarcely sufficient as yet for a pronouncement.

Taking all things into account, choke cherry would appear to be the species most usually present where potato growing would be affected. In some of the lighter soil regions bird cherry would partly take its place. *Prunus nigra* might often be an additional factor.

From the standpoint of geographical distribution and frequency these are the three most abundant species in potato-growing regions. It would now be most interesting to learn whether these species or perhaps the less common species are of most importance as overwintering hosts of the vectors.

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EFFECT OF NUMBERS OF COLONIES PER PLATE ON THE ESTIMATE OF THE BACTERIAL POPULATION IN SOIL[1]

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Abstract

Numbers of colonies of bacteria on plates from two series of 36 plots were found to vary widely on one date of sampling and from day to day. In an attempt to determine the effect of this variation and of introducing a dilution of different strength during the progress of an experiment on the accuracy of estimates, samples were plated in a series of replicate dilutions, with three parallel plates from each. In Experiment 1, four replicate dilutions of one strength were used. The experiment was repeated 120 times and shows that parallel dilutions, prepared by a slightly altered technique, add only very little to the error of the experiment beyond that of the interaction. In Experiment 2, three replicate dilutions of 1 : 300,000; 1 : 400,000, and 1 : 500,000 strengths, respectively, were used. This experiment was repeated 162 times, yielding a final result similar to that from dilutions of one strength. In the third experiment three replicate dilutions of 1 : 150,000; 1 : 250,000, and 1 : 400,000 strengths, respectively, were used. This experiment was repeated 360 times and shows that parallel dilutions, varying to this extent, introduce an appreciable error to the experiment.

When counts from the 1 : 150,000 dilutions were correlated with those from the 1 : 400,000 dilutions of the same samples, it became obvious that the two systems of diluting do not provide estimates that are interchangeable. This proved true also with counts from five other pairs of dilutions, prepared in Experiments 2 and 3. In each case a curve was fitted to the data from the correlation surface, resulting in a sixth degree curve for the 1 : 150,000 and 1 : 400,000 dilutions, third degree curves for four other pairs of dilutions, and a linear relation for one pair.

The results show that one dilution does not provide an estimate that agrees with that from another dilution of different strength, except at one count. A system of making estimates by using two dilutions and correcting the counts to provide interchangeable estimates is suggested.

The data from Experiment 3 were corrected on this basis and analysed. The corrected data yield an F value for dilutions of different strength about the same as that obtained by the use of parallel dilutions of one strength.

Introduction

In a long term investigation designed to study, among other things, the effect of various cultural practices and fertilizer treatments on the numbers of micro-organisms in soil in relation to the development of root rots in cereals, the averages of four counts from each of 36 plots obtained on one day were found to vary as much as threefold at some platings. Furthermore, the counts varied appreciably at different samplings, yielding averages ranging

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Contribution from the Department of Bacteriology and Animal Pathology, the University of Manitoba, Winnipeg, Man., with financial support from the National Research Council of Canada.
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[3] *Research Assistant in Bacteriology.*

from 10 to 150 colonies per plate in each season. The higher count is lower than the accepted upper limit in certain fields of study. Still, the probability that the number of colonies developing on plates from such a complex population as is found in soil may affect the accuracy of the estimate must be considered. Too many colonies might introduce the factor of crowding, with consequent scarcity of limiting food material, or mutual relations antagonistic or stimulative. On the other hand, too wide separation of colonies might lead to the lack of stimulation among certain types, resulting in failure to grow and too low a count. Errors attributable to such phenomena might alter the final estimate to such an extent as to conceal real effects in the problem under consideration and lead to unsound conclusions. Accordingly, it appeared advisable to consider the error of estimates from counts varying so widely and the effect of introducing a different dilution in the progress of an experiment in order to provide counts per plate falling within a definite range.

Experimental

Before attempting to determine the effect of introducing a dilution of different strength into an experiment, it appeared necessary to improve the technique so as to reduce the error of parallel dilutions of one strength, or at least to determine the variance attributable to parallel dilutions under the conditions imposed by the technique. Recently, the authors (2) showed that the technique, as commonly used, provides significant differences among dilutions raised in parallel series from one first dilution, and improved the system of diluting appreciably by starting with a 1 : 50 first dilution. After a slight change in procedure, which will be considered in a later report, was adopted, a complex experiment was carried out to obtain information on several points simultaneously. Consequently, data are available from 120 fifty-gram portions of soil diluted 1 : 50 in water. Four dilutions from each original 1 : 50 dilution were raised in parallel series to the 1 : 400,000 dilutions used for plating. Each final dilution was plated in three parallel plates. Then the actual counts were corrected to provide estimates per gram of dry soil. One test supplied estimates as follows:

	Millions of colonies per gram of dry soil		
	Plate 1	Plate 2	Plate 3
Final dilution 1	25.38	24.32	23.80
Final dilution 2	21.68	25.38	30.67
Final dilution 3	27.50	27.50	29.61
Final dilution 4	24.32	30.67	28.56

The experiment provides 120 such tests, each with 3 D.F. for parallel dilution 2 D.F. for the estimation of the error of systematic order of plating, and 6 D.] for interaction or a measure of the inconsistencies of the data in each tes

This interaction may be considered the error appropriate for testing the effect of steps in the technique of the plating method.

In this type of experiment one is not concerned with measuring effects in terms of 5% and 1% levels of significance, but rather with obtaining F values close to 1.00 when the number of degrees of freedom for error is large. In this case, the F value is the variance for dilutions divided by that for interaction. The variance for a main effect includes the variance for interaction and a portion added by the effect under consideration. Thus, as the values approach 1.00 the portion added by the effect tested approaches zero. The pooled data from the 120 tests give the following results:

Source	D.F.	Variance	F	5% Pt.
Replicate dilutions	360	20.3919	1.1077	1.22
Systematic order	240			
Interaction	720	18.4090		

Since the experiment involves a large number of D.F. and the F value is close to 1.00, this is evidence that parallel dilutions add only very little to the error of the experiment beyond that of the interaction.

The second experiment was similar to the first except that the three dilutions raised in parallel series gave final dilutions of 1 : 300,000, 1 : 400,000, and 1 : 500,000, respectively. After the counts were corrected to provide estimates per gram of dry soil, the data were analysed as in the previous experiment. The pooled results of 162 tests, each made up of three estimates by each of the three systems of diluting, are presented below.

Source	D. F.	Variance	F
Replicate dilutions	324	11.6776	1.1065
Systematic order	324		
Interaction	648	10.5536	

This finding is in close agreement with that of the previous experiment and shows rather clearly that changes in dilutions, within the range considered, add very little to the error of the experiment beyond that added by parallel dilutions of one strength.

The third experiment was designed to test the effects of a wider range in dilutions, but otherwise was the same as the second. The final dilutions were 1 : 150,000, 1 : 250,000, and 1 : 400,000 respectively. The samples considered in this study provided estimates ranging from 10 to 20 millions per gram of dry soil. On this basis the three dilutions from the 20 million population should give counts of 133, 80, and 50 colonies per plate, respectively.

The data represent estimates corrected to the number per gram of dry soil on 360 tests with nine plates each.

Source	D.F.	Variance	*F*
Replicate dilutions	720	16.6258	2.2697**
Systematic order	720		
Interaction	1440	7.3414	

In this case there is evidence that the use of three dilutions, varying to such an extent, introduces an error to the experiment appreciably greater than that added by parallel dilutions of one strength. Further, data not included indicate that differences among counts from this range of dilutions vary from day to day. Since the analysis for each day's plating was based on 324 plates, this finding eliminates the possibility of obtaining a satisfactory correction by averaging estimates obtained from the three dilutions.

The actual counts obtained by means of the three systems of diluting varied within a comparatively narrow range during the progress of the experiment. Accordingly, it would be expected that the estimates based on the different systems of diluting would be interchangeable. To determine this, a correlation surface was prepared, using the means of each set of three counts obtained from the 1 : 400,000 dilutions as x and the corresponding means of sets obtained from the 1 : 150,000 dilutions as y. The values of x ranged from 22 to 54 and, for convenience in fitting the curve, were divided into 17 classes with class intervals of two. Those for y ranged from 55 to 130. In this case the class interval was one. The mean of the y values placed in each class of x was calculated. These 17 means were plotted. The theoretical count for each dilution was determined on the basis of a population producing from 10 to 20 million colonies per gram of dry soil. The results made it obvious that the data from the two systems of diluting do not provide estimates that are interchangeable. Counts of 70 obtained from the 1 : 150,000 dilution were approximately 10% too high as compared with the theoretical. Those of 100 from dilutions of the same strength were about 40% too low. In order to determine whether this type of relation among counts obtained from different dilutions was consistent, a similar procedure was followed using 1 : 200,000, 1 : 250,000, 1 : 300,000, and 1 : 500,000 dilutions as y respectively, with the 1 : 400,000 dilution as x; and also counts from the 1 : 300,000 against those from the 1 : 500,000 dilutions. Data for these tests were obtained from Experiments 2 and 3. The results show a similar trend in all cases except those including the 1 : 500,000 dilutions. Contrary to expectation based on the phenomenon of crowding, estimates from the 1 : 500,000 dilutions were slightly lower, rather than higher, than those from the 1 : 400,000 dilutions.

The data do not appear to follow a linear relation. Accordingly, they were coded and in each case a curve was fitted, following the method for fitting

polynomials outlined by Goulden (1). The calculations in the table on page 230 were carried out according to directions given on page 84. Third degree curves were obtained in all except the 1 : 150,000 with the 1 : 400,000 dilutions, in which case a sixth degree curve gives the best fit. The 1 : 500,000 and 1 : 400,000 dilutions give a linear relation. The following note was provided by Dr. Goulden:

"In the calculation of the polynomial equation the actual class values were replaced by the natural numbers beginning with 1. To deduce the actual equation it was necessary, therefore, to decode. If the calculated equation is:

$$Y' \text{ equals } c_0' + c_1'x' + c_2'x'^2 + c_3'x'^3$$

and if i_y and i_x are the actual class intervals of y and x, then the actual equation is:

$$Y \text{ equals } y_0 + i_y\left(c_0' + c_1'\left(\frac{x - x_0}{i_x}\right) + c_2'\left(\frac{x - x_0}{i_x}\right)^2 + \ldots\right)$$

where y_0 and x_0 are chosen so that

$$\left(\frac{y_1 - y_0}{i_y}\right) \text{ equals } \left(\frac{x_1 - x_0}{i_x}\right) \text{ equals } 1$$

y_1 and x_1 being the first of the actual class values of y and x."

The results are shown in Fig. 1. It may be noted that the curve intercepts the line for the theoretical at about the same point in each case, and that the counts give estimates that are interchangeable only at this point of intersection. Thus, the greater the difference in count from that of the point of intersection, the greater the discrepancy between the actual and theoretical. In each case the curve at the left of the intersection shows a tendency for the counts plotted as y to be too high in comparison with the theoretical. Counts of the same size to the right of the point of intersection are lower than the theoretical. This would appear to be an inconsistency if it were not for the fact that dilutions of the same strength were taken as x in each case. The discrepancy apparently results from the fact that the counts taken as x values become too low when there are too few colonies in the plate.

These results provide convincing evidence that dilutions of one strength fail to give any indication of the errors of estimates produced by crowding of colonies on plates, on the one hand, and too wide separation of colonies on other plates, on the other. Neither do they furnish any clue as to the number of colonies per plate that is least affected by these factors under the conditions of an experiment. Obviously, that number might not be the same under different soil conditions.

Since it is not possible to predict the range in counts likely to be encountered in an extended experiment, and, likewise, since the range from dilutions of one strength may be so wide as to produce serious errors in some of the estimates, there appears to be no alternative other than to suggest the use of two dilutions of different strengths and the application of a correction to adjust estimates based on the counts affected most seriously by this plate factor error. This

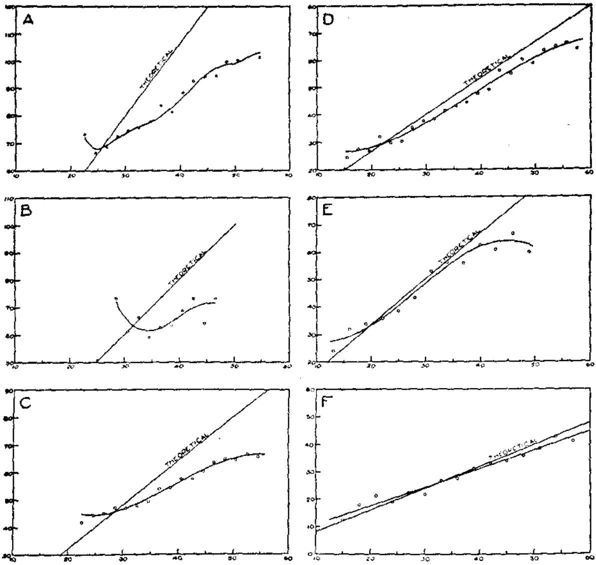

FIG. 1. *Plate counts of soil bacteria from samples plated simultaneously in two dilutions of different strengths. Dots represent means of y values placed in each class of x. Curves fitted according to the polynomial equation. Straight lines represent theoreticals.*

A. 359 pairs of dilutions; y = 1/150,000 and x = 1/400,000
B. 36 pairs of dilutions; y = 1/200,000 and x = 1/400,000
C 323 pairs of dilutions; y = 1/250,000 and x = 1/400,000
D. 159 pairs of dilutions; y = 1/300,000 and x = 1/400,000
E. 159 pairs of dilutions; y = 1/500,000 and x = 1/400,000
F. 159 pairs of dilutions; y = 1/300,000 and x = 1/500,000.

involves the preparation of a curve similar to the curves in Fig. 1, and from it a simpler correction curve similar to the curves in Fig. 2. The latter represent the values for the curves of Fig. 1 plotted as deviations from the theoreticals based on interchangeability of dilutions. Either curve will show the point of intersection, or the neutral count for each dilution where a correction is unnecessary.

For simplicity in explaining the method of correcting, the low count dilution is referred to as dilution X and the high count dilution as dilution Y. If a given sample has an X dilution count that is higher than that at the neutral point, this count is used without correction. The Y dilution count on the

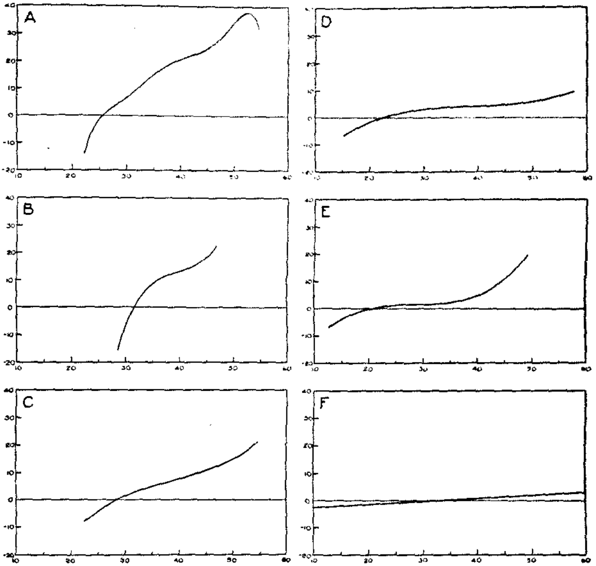

FIG. 2. *Correction curves for counts from two dilutions of different strengths, derived from the data of Fig. 1. The 0 line represents the theoreticals for y.*

same sample is corrected to make interchangeable with X dilution counts. To make the correction, find the X dilution count on the base line and at this point measure the difference from the curve for Y to the theoretical. Add the difference to the actual count for the Y dilution of the sample. The estimate on the sample is obtained by averaging that calculated from the actual count for dilution X and that from the Y dilution count, corrected as above. This correction lessens the error attributed to crowding in the plates with the larger number of colonies. Since it has been shown that counts from the X dilution are too low when less than that at the neutral point, the Y dilution count is used without correction in a sample that has a low X dilution count and the latter is corrected to make interchangeable with Y dilution counts. This involves the preparation of a second curve, where the high count dilution is considered as X and the low count dilution values are plotted. The procedure for correcting is the same as in the former case. Here, the correction lessens the error attributed to the lack of mutual stimulation among colonies in plates from the dilution giving the lower counts.

As a test of the effectiveness of this correction, the means of the counts n the original data from Experiment 3 were adjusted and analysis of variance vas run on the corrected data. The result follows:

Source	D.F.	Variance	*F*
Replicate dilutions	720	7.7307	1.0530
Systematic order	720		
Interaction	1440	7.3414	

The variance for dilutions is reduced from 16.6258 shown on the uncorrected data to 7.7307, with a corresponding decrease in the *F* value. The *F* value on the corrected data compares favourably with that of 1.1077 shown previously from Experiment 1 and that of 1.1065 from Experiment 2. These results furnish strong evidence that the correction was appropriate and reduced differences deliberately introduced by the system of diluting to approximately those obtainable among dilutions of one strength.

Additional evidence that the factor to which this correction was applied had a tendency to mask real effects in the data may be demonstrated as follows. In Experiments 1, 2, and 3 after the correction was applied, the variance introduced by replicate dilutions is only 5 to 10% larger than that due to the interaction of dilutions and systematic order. Therefore, for purposes of illustration, the dilution variance may be used as error instead of the interaction. The 36 tests for each day's plating represent attempts to measure differences among replicate field samples. *F* values calculated by dividing the variance available for estimating the effect of field samples by the dilution variance as error, give the following results on the original data and on the same data after the correction was applied.

Original data	Corrected data
1.6458	5.0340
2.2376	13.4925
.6769	6.4460
.9241	4.7326
1.2544	5.7404
1.1913	6.2913
1.5711	4.3419
1.4273	7.7981
3.9320	8.6872
2.6615	14.3237

For 35 and 72 D.F.

5% Pt. 1.59
1% Pt. 1.92

It may be observed that in the original data only one value is over that at the 5% level of significance and three over that at the 1% level of significance, while the lowest value in the corrected data is more than double that at the 1% level of significance. Consequently, when the data are corrected to the point where estimates for the three dilutions are interchangeable, the possibility of finding differences among field samples is much greater. Thus, this

correction reduces the error pertaining to the number of colonies per plate in the technique and makes the results obtained considerably more sensitive as potential indicators of differences among samples.

Discussion

The finding that a high count, although well within the range of accepted numbers of colonies per plate, introduces an error in the estimate that is greater than that of a low count from a dilution of the same strength further complicates the problem of evolving a satisfactory technique for determining the number of bacteria in soil. It indicates that the number of colonies that grow in a limited quantity of medium and under a definite set of conditions is controlled by what may be termed a plate factor error. A count of 30 may be subject to a plate factor error that is of minor significance; one of 40 to an error that is more serious and one of 80 to one that is more than double that of the 40 count. This makes it apparent that with counts of the order of 100 per plate an effect under consideration may be masked by the plate factor error to a greater extent than with counts of 40.

While the result obtained was limited to the mixed population developing from soil of one area under the conditions of the experiment, it is conceivable that the same phenomenon may apply in relation to counts of bacteria in other products. For example, a similar test might show that a count of 300 colonies from one dilution of milk may not be more than half what it would have been had the dilution used given a count of 30 colonies per plate. This would mean that the sample with the higher number of colonies per plate has the advantage in a product whose quality is measured in terms of low counts.

Likewise, the failure of two dilutions of different strengths, although such dilutions provide data relatively similar in the light of accepted ranges in counts, to provide estimates that check presents a problem of serious concern to one interested in estimating the soil population for the purpose of studying the relation of numbers of bacteria to conditions or treatments in terms of recognized statistical significance. Since the proper dilution for a given soil under one set of conditions is not predictable, the use of two dilutions of different strengths not only allows for the application of the correction suggested, but provides two chances of obtaining counts within the range most desired. This does not mean that the dilutions should differ widely. Undoubtedly, a wide range in dilutions would introduce the effect of extremes in either low or high counts or both. Neither should the dilutions represent those giving counts too closely alike, since it is conceivable that the disturbance resulting from the number of colonies per plate would be similar in both dilutions. The use of dilutions yielding counts varying as 1 : 2 or 1 : 3 would seem to be most desirable. Under the writers' conditions, the dilutions giving the lowest number of colonies should be such as to provide counts above 25 per plate. The regression line for the 1 : 500,000 dilution counts as y and the 1 : 400,000 counts as x appears to provide further information on the effect of too low counts per plate. In all other cases, the lower dilutions, with

higher counts than those of the 1 : 400,000 used as x, give counts that become increasingly lower than the theoretical as the size of the counts increases. In this instance, the opposite appears to be the case. The higher dilution with the lower actual counts produces results that are lower than the theoretical. The fact that the 1 : 500,000 dilution counts may be too low on the basis of the theoretical may provide an explanation for the relatively small correction shown for the 1 : 300,000 counts when the 1 : 500,000 dilution counts are used as x.

The relation of the number of colonies that grow in a plate to the probable number of organisms actually in the portion measured provides reason for considering the influence of associative action among types of organisms on the number of colonies that develop. In the low count plates, the organisms depend on the food ingredients provided, while in the medium count plates, conceivably, the organisms may be affected by stimulation from the products released by other colonies near by and as well, by probable antagonistic relations. In crowded plates, because of the proximity of colonies and the greater probability of the presence of antagonistic types, the suppressing effect of antagonism may mask any stimulative effect caused by other colonies and result in a count much lower than the theoretical.

The correction suggested may find application in the study of antagonism between definite species of micro-organisms. The result of antagonistic relations could be studied quantitatively by making estimates of the two species developing separately and of the two in different proportions developing together on plates. Thus, it would be possible to measure the relative antagonistic effect of different species against one test organism.

Finally, the use of dilutions providing two ranges of counts and the correction suggested provides a method for estimating a population with a greater degree of accuracy than could result from the use of one dilution only. This procedure involves the replication of dilutions, with added labour, but results in an estimate of greater value without increasing the number of plates prepared. Three plates from each of two dilutions of different strengths would provide the basis for an estimate of greater value for the population considered than would six plates prepared from one dilution.

Acknowledgments

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THE INFLUENCE OF LIGHT AND CERTAIN OTHER ENVIRONMENTAL FACTORS ON THE MATURE-PLANT RESISTANCE OF HOPE WHEAT TO STEM RUST[1]

By T. Johnson[2] and Margaret Newton[3]

Abstract

Experiments have been conducted to determine the degree to which the mature-plant reaction of Hope wheat to race 21 of *P. graminis Tritici* Erikss. and Henn. is affected by variation in light intensity, length of daily light period, temperature, soil moisture, and supply of mineral nutrients. All these factors gave evidence of influencing rust reaction. A 60% reduction in light intensity during the whole growing period of the plants tended to influence their rust reaction in the direction of increased susceptibility and caused a modification in the morphology of the plants in that they were softer, less rigid, and higher in moisture content than plants grown in full daylight. The shift towards susceptibility was, however, not very marked, and in only one experiment did the reaction of the plants approach complete susceptibility. Reduction in length of daily light period also influenced the rust reaction of the plants towards susceptibility. Plants receiving six hours of light daily were less resistant than plants receiving ten hours of light and these, in turn, were slightly less resistant than plants receiving full daylight for the period of the experiment (February to June). Experiments on the effect of temperature on the reaction of Hope wheat have shown that constant high temperature (75° to 80° F.) is capable of causing a partial or even complete breakdown of mature-plant resistance. Some evidence was secured that abundance of soil moisture and of mineral nutrients diminished rust resistance.

Introduction

In recent years the varieties Hope and H 44-24 have been utilized to a great extent in crosses designed to develop rust-resistant varieties of common wheat. These varieties, though susceptible to many physiologic races of stem rust in the seedling stage, appear to be highly resistant to most—if not all—races in the mature-plant stage. This type of resistance has frequently been termed "mature-plant" resistance. As the new varieties developed through crosses with Hope and H 44-24 also exhibit this type of resistance, it is obviously of much importance to determine its stability under the various environmental conditions to which these new varieties will inevitably be subjected when they become widely distributed. The necessity for a study of this kind is emphasized by the fact that varieties possessing mature-plant resistance do not by any means exhibit the same degree of resistance in all regions where they, thus far, have been grown.

On the North American continent varieties with mature-plant resistance have, in general, proved highly resistant. Abbott (1), however, has reported that, when grown in Peru, South America, on irrigated soil, Hope and Pentad

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rusted heavily. Similarly, when grown in Kenya, East Africa, varieties with mature-plant resistance have shown considerable rust development (2, 3). The possibility that the susceptibility of these varieties is due to the presence of physiologic races differing from those prevalent in North America cannot be entirely overlooked. In Kenya, however, where surveys of physiologic races have been conducted annually, three of the four races known to exist when these wheats were tested, races K_1, K_2, and K_3, are identical with races 21, 17, and 34, to which Hope, H 44, and their derivatives are highly resistant in Canada and the United States (9).

While it is believed that the environment in the above-mentioned regions has greatly influenced the stem-rust reactions of these wheat varieties, it is not at all clear what factors in the environment have exerted the greatest influence. The two localities in which these tests were conducted, Lima, Peru, and Njoro, Kenya, do not appear to have many environmental conditions in common except that, as both are located close to the equator, day and night are approximately equal in both places. At Lima the altitude is low, about 500 ft., the rainfall negligible so that wheat must be irrigated, while fogs and mists are common during the period when wheat is grown. At Njoro the altitude is high, about 7,100 ft., while the annual rainfall varies between 30 and 50 in. In both localities temperatures are moderate during the growing season for cereals.

As suggested by Burton (*cf.* Hart and Zaleski (5, p. 1044)), altitude is probably of little importance except in so far as it modifies other environmental conditions. The approximate equality of day and night may be of some importance in modifying plant growth and reaction to rust. Whatever the conditions responsible, it is, at any rate, evident that in Kenya the growth of wheat is much slower than in Canada. Hope wheat sown on April 24, 1935, was not harvested until October 7—166 days after sowing*. An average for a period of six years showed that, at Winnipeg, the same variety matures in 90 days. In Peru, the fact that the plants were grown under conditions of irrigation, accompanied by fogs and mists during the growing period, may have played a part in breaking down mature-plant resistance.

Hart and Zaleski (5) have already studied the effect of light intensity and temperature on the reaction of Hope wheat to stem rust. They have found that, under reduced intensity of light, Hope wheat becomes susceptible to race 21 of wheat stem rust. High temperatures (26° to 33° C.) on the contrary tended to increase the resistance of the plants. Apart from their work no serious attempts appear to have been made to evaluate, by experimental means, the relative influence of different factors of the environment on mature-plant resistance.

The experiments described in the present paper were conducted with the object of discovering, if possible, the relative importance of such environmental factors as intensity of light, duration of daylight, temperature, soil

* *Information furnished privately by Mr. R. J. Lathbury, Plant Breeding Station, Njoro, Kenya*

moisture, and supply of soil nutrients, in breaking down mature-plant resistance. The experiments were carried out in the greenhouse as these factors could be more readily controlled under greenhouse conditions than in the field.

Method of Estimating Degree of Resistance

In the work reported in the present paper it was essential to secure as accurate a comparison as possible of the resistance to rust of a given variety grown under different environmental conditions. In most of the experiments the infection types occurring on the plant were recorded in detail, leaf by leaf and internode by internode. As different parts of the same plant commonly bore infections of two or more types, difficulties were often experienced in deciding on an infection type that could be considered characteristic of the variety under a given environmental condition. An attempt was made to overcome these difficulties by utilizing the following scale of numerical values assigned by Goulden, Newton, and Brown (4) to the various infection types originally described by Stakmen and Levine (13).

Numerical values	1	2	3	4	5	6	7	8
Infection types	0	0;	1=	1−	1	1±	1+	1++
Numerical values	9	10	11	12	13	14	15	16
Infection types	2=	2−	2±	2+	2++	3=	3−	3
					x−	x	x+	x++
Numerical values	17	18	19	20	21	22	23	24
Infection types	3±	3+	3++	4=	4−	4	4+	4++

By assigning numerical values to all the infection types recorded for a plant or a group of plants of the same variety, it was possible to arrive at a mean numerical value indicative of the average rust reaction of the variety. Resistance is indicated by numerical values below 13 and susceptibility by values above 16. The intermediate values 13 to 16 represent the transition from resistance to susceptibility.

The Effect of Reduced Light Intensity on the Reaction of Hope Wheat to Race 21 of Stem Rust

Three separate greenhouse tests were carried out to determine the effect of reduced light intensity on rust development on the variety Hope. The first of these was conducted in the spring (March and April) of 1936, the second in the early winter (November and December) of the same year, and the third in the spring of 1937 (April and May). The conditions of light were approximately the same in the first and third tests, but in the second the day was shorter and the light intensity lower.

The reduction of light intensity was effected by growing the plants from the seedling stage to maturity under a covering of cheesecloth, one ply being used in the first test and two in the second and third. After inoculation, the plants were again exposed to the light conditions under which they were grown.

The same race of stem rust, race 21, was used in all three trials. As the shaded plants used up less moisture than the non-shaded, attempts were made, in all the experiments, to equalize soil moisture in the shaded and non-shaded pots by watering the two lots in such a way that the soil in both maintained about the same appearance and handling qualities.

In the first test ten 6-in. pots, each containing three plants, were placed under a one-ply cheesecloth cover soon after the emergence of the seedling plants, while ten pots were kept in the open as controls. About seven weeks after planting, the plants in two pots of each group were inoculated with race 21 by applying the urediospores to the leaf and stem surfaces with the fingers. Thereafter, at intervals of about ten days, two pots were removed from each of the two groups and the plants inoculated in the same manner. The first inoculations were performed on March 13, when the plants were in the early shooting stage, the last on April 24, when the plants were heading.

TABLE I

THE EFFECT OF REDUCED LIGHT INTENSITY ON THE REACTION OF HOPE WHEAT TO *P. graminis Tritici*, RACE 21

The wheat was planted January 24, 1936, and inoculated at different dates between March 13 and April 24. (The upper half of the table shows the degree of resistance in terms of infection type; the lower half shows the degree of resistance in terms of numerical values calculated from the scale developed by Goulden, Newton, and Brown.)

Date of inoculation	Date of marking	Leaves		Stems	
		Shaded	Not shaded	Shaded	Not shaded
13-3-36	27-3-36	2 to 4=	2 to 3	2 to 4−	3
24-3-36	11-4-36	2 to 4−	0; to 4	3±	3 to 4
3-4-36	23-4-36	1 to 3=	2 to 3	2 to 3	2 to 3−
17-4-36	2-5-36	1 to 2	1 to 2	2 to 3+	1 to 3
24-4-36	8-5-36	0; to 1+	0; to 2	1 to 3	1 to 3
13-3-36	27-3-36	15.4	13.7	16.3	16.0
24-3-36	11-4-36	16.0	13.2	17.0	19.0
3-4-36	23-4-36	9.5	13.5	13.5	12.6
17-4-36	2-5-36	8.0	7.5	14.4	10.6
24-4-36	8-5-36	4.2	6.0	10.5	10.2
Mean of all tests		10.6	10.8	14.3	13.7

The results of this test, which are expressed in Table I, do not reveal any appreciable difference in the type of infection produced by race 21 under the two different conditions of light. Under both full and reduced light there was a progressive increase in the resistance of both leaves and stems with a progressively later date of inoculation. This is probably due mainly to the increasing age of the plants, as each successive batch of plants was about 10 days older than the preceding one. The possibility, however, is not altogether excluded that the increasing length of day and intensity of daylight may have played some part in the development of greater resistance.

As it was thought possible that the failure of the first test to show any relation between light intensity and rust reaction might have been due to an insufficient reduction of the intensity of light, it was decided to conduct a somewhat similar experiment with Hope wheat at a time of year when the intensity of daylight was lower, and with the plants shaded by two ply of cheesecloth instead of one as in the earlier test.

Twenty-four 6-in. pots planted on September 4 were divided into two equal lots, one of which was exposed to natural daylight conditions while the other was placed in a cage covered by two ply of cheesecloth. Between November 21 and 26, when the plants were in the boot stage, they were inoculated with race 21. Following inoculation the shaded plants were again placed under their cheesecloth cover. Notes made on rust development on December 11 are summarized by means of numerical values in Table II.

TABLE II

THE EFFECT OF REDUCED LIGHT INTENSITY ON THE REACTION OF HOPE WHEAT TO *P. graminis Tritici* RACE 21

The wheat was planted September 4, 1936, and inoculated between November 21 and 26

Pot	Reaction in terms of numerical values for infections			
	Leaves		Stems	
	Shaded	Not shaded	Shaded	Not shaded
1	10.5	13.5	16.0	16.5
2	14.5	—	16.5	13.5
3	16.0	13.5	16.5	13.5
4	16.0	5.0	13.5	11.5
5	16.0	—	16.5	10.0
6	14.5	11.0	14.5	10.5
7	16.0	13.5	16.0	8.0
8	13.5	13.5	13.0	10.5
9	16.5	19.0	18.0	13.5
10	13.5	—	16.5	10.0
11	10.0	14.5	16.0	11.5
12	13.5	16.5	16.5	13.5
Mean	14.2	13.3	15.8	11.9

It was obvious from a cursory examination that the shaded plants were somewhat more susceptible than the non-shaded. The percentage of infection was also considerably higher on the shaded plants: the average of the estimates for the individual plants were 5.4% on the leaves and 11.3% on the stems of the shaded plants as against 0.7% and 6.0% for the leaves and stems of the non-shaded plants. Although the shaded plants were noticeably more susceptible than the non-shaded ones, the average increase in susceptibility was not very striking. Translated from numerical values to infection types, it amounted to a shift from 2++ and x− to 3= and x on the leaves; and from 2+ to 3 and x++ on the stems.

The shaded and non-shaded plants differed not only in their reactions to rust but also in certain other characteristics. The non-shaded plants were harsher to the touch and considerably more rigid in both stem and leaf than the shaded ones; this gave the impression of somewhat greater succulence in spite of their more slender culms. This observation led to a determination of the moisture content of the two sets of plants. The moisture content of the shaded leaves was 82.68% as against 76.62% for those of the non-shaded; and of the shaded stems, 85.46% as against 81.59% for the non-shaded.

It can scarcely be doubted that the increased succulence and the greater susceptibility of the shaded plants are related phenomena. There is some doubt, however, as to whether or not the greater succulence of the shaded plants was entirely the result of the lower light intensity at which they were grown. It should be noted that the relative humidity within the cheesecloth cage averaged almost 10% higher than outside. Nevertheless the difference in relative humidity inside and outside the cage was slight as compared with the difference in light intensity. Measurements with a Weston Photronic Exposure Meter showed that, under conditions of diffuse daylight, the double ply of cheesecloth transmitted only from 35 to 40% of the light. As the experiment was conducted in the fall and early winter, even the plants receiving

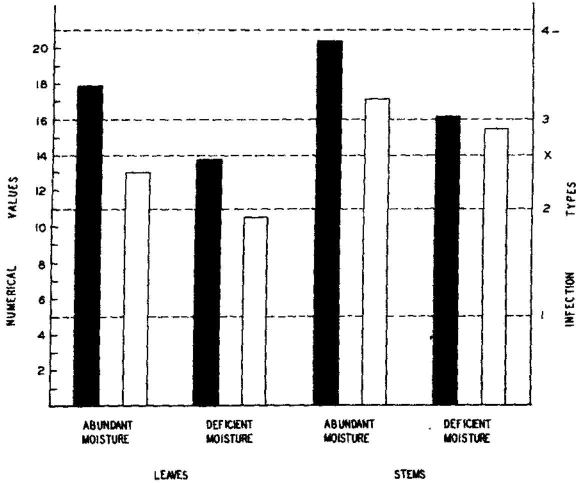

FIG. 1. *Average rust reactions of shaded and non-shaded plants of Hope wheat grown under conditions of abundant and deficient soil moisture. The black columns represent shaded, the white columns, non-shaded plants. The plants were inoculated during the third week of April.*

full daylight grew under distinctly subnormal light conditions. Despite the still lower light intensity received by the shaded plants, their resistance to race 21 was broken down to only a rather limited extent.

A third experiment of a similar type was carried out in the spring of 1937, the plants being sown late in January. Twenty-four pots containing three plants each were divided into two groups one of which was shaded by two ply of cheesecloth while the other was exposed to ordinary daylight. This experiment, however, differed from the previous ones in that one-half of the shaded and non-shaded plants, respectively, were grown under conditions of deficient soil moisture. The purpose of this modification was to reduce the succulence of both shaded and non-shaded plants and determine the relation of such reduction in succulence to rust reaction.

The plants were inoculated with race 21 at the time of the emergence of the heads, in the third week of April. After inoculation each group of plants was re-exposed to its previous conditions until notes were taken on rust reaction. The results of this experiment are expressed in Table III and Fig. 1.

TABLE III

THE EFFECT OF REDUCED LIGHT INTENSITY ON THE REACTION OF HOPE WHEAT TO RACE 21 OF *P. graminis Tritici* UNDER CONDITIONS OF ABUNDANT AND DEFICIENT MOISTURE

The wheat was planted late in January, 1937, and inoculated in the third week in April

Treatment	Pot	Reaction in terms of numerical values for infections			
		Leaves		Stems	
		Shaded	Not shaded	Shaded	Not shaded
Plants supplied with abundant moisture	1	18.5	14.0	19.0	18.0
	2	17.0	15.0	19.0	17.0
	3	19.0	13.0	21.0	17.5
	4	19.0	11.0	21.0	15.0
	5	17.0	—	21.0	20.0
	6	17.0	12.0	21.0	15.0
Mean		17.9	13.0	20.3	17.1
Plants subjected to drought	7	13.5	6.0	17.0	17.0
	8	16.0	12.0	17.0	15.5
	9	16.0	11.0	18.0	16.0
	10	11.0	11.0	13.5	15.5
	11	13.0	11.0	17.0	15.5
	12	13.5	12.0	15.0	13.0
Mean		13.8	10.5	16.2	15.4

The effect of shading on the plants, and particularly those receiving abundant moisture, was very similar to that noted in the preceding experiment. Moisture determinations on leaves and stems of the plants receiving abundant moisture showed, at the finish of the experiment, about two weeks after heading, that the shaded leaves contained 77.93% moisture as against

72.20% for the non-shaded leaves, while the shaded stems contained 77.36% moisture as against 67.82% for the non-shaded.

The infection results, as summarized in Fig. 1, show that both light and moisture were capable of affecting the rust reactions to some extent. In general, the susceptibility of both leaves and stems was somewhat greater than in previous experiments. Resistance was greatest under conditions of full light plus deficient moisture, whereas susceptibility was greatest under conditions of reduced light plus abundant moisture. Under the latter conditions the plants would have to be described as moderately susceptible. The relation between succulence and susceptibility was quite evident.

The Relation of Light to Regional Susceptibility

It is possible that light conditions may account for the "regional susceptibility" of varieties possessing mature plant resistance to which attention has been called by Goulden *et al.* (4), Popp (12), and Newton and Brown (11). It is characteristic of varieties with mature-plant resistance that the growing plant tissues are susceptible to rust while still covered by the sheaths, but acquire resistance very soon after they become exposed. Before they become exposed, these tissues are protected from light to a certain extent by the overlying sheaths. An attempt was made to gauge the extent of this protection by determining by means of a Weston Photronic Exposure Meter the proportion of diffuse daylight transmitted by freshly detached sheaths of wheat plants approaching the boot stage of growth. The sheaths were found to transmit from 25 to 33% of the light. Under identical light conditions, the two-ply cheesecloth used for shading plants transmitted 40% of the light while the cotton cloth used in some experiments for the same purpose transmitted 29%. It would seem therefore that the sheaths afford much the same degree of protection from light as the materials used for shading plants in the experiments reported in this paper. Whether the protected parts are susceptible because they are shaded from light, or because of the higher humidity within the sheath, or for other reasons, does not appear to have been proved experimentally. As it has been shown by Johnson and Johnson (7) that the immature tissues within the sheaths are very much richer in reducing sugars than are the fully developed tissues, and as these sugars may be regarded as the structural units for further tissue development, it is quite possible that the greater susceptibility of the less mature tissues is related to their stage of development rather than to deficiency in light.

An Experiment to Determine the Effect of Different Periods of Daylight on Stem-rust Development on Hope Wheat

The idea of carrying out an experiment on the effect of different periods of daylight on mature-plant reaction was suggested by the results of certain tests in Kenya, East Africa, of wheat varieties developed at the Dominion Rust Research Laboratory. These varieties, which in Western Canada exhibited a high degree of mature-plant resistance, showed a considerable

degree of susceptibility when grown at Njoro in Kenya, in 1935. Environmental conditions in Kenya are, of course, strikingly different from those prevailing in Western Canada. As Njoro is on the equator, where day and night are approximately equal, the period of daylight is considerably shorter than that prevailing in Western Canada during the growing period where the period of longest daylight exceeds 16 hr. As a result of the shorter period of daylight, and perhaps other conditions as well, the growing period for wheat in Kenya is at least two months longer than in Western Canada.

It was thought possible that a greenhouse experiment, in which both temperature and period of daylight were kept under strict control, might produce information on the effect of length of day on mature-plant resistance. Accordingly, the variety Hope was grown from seedling stage to maturity under conditions of three different periods of daylight: a 6-hr. period, a 10-hr. period, and the full period of daylight during the course of the experiment. The last-mentioned varied considerably as the experiment was begun at the beginning of February and was not completed until June. As it was essential to keep conditions other than light identical for all the test plants, these were

FIG. 2. *The effect of different periods of daylight and of different temperatures on the development of Hope wheat. The photograph was taken about three months after planting.* 1—*10 hr. light daily; 60 to 61° F.* 2—*6 hr. light daily; 60 to 61° F.* 3—*Normal daylight; 10 hr. daily at 60 to 61° F. and 14 hr. at 75 to 80° F.* 4—*Normal daylight; 60 to 61° F.* 5—*Normal daylight; 75 to 80° F.*

grown (in 6-in. pots) in the same greenhouse, maintained at a temperature of 60° to 61° F. The plants exposed to daylight for 6 and 10 hr. respectively were kept during the remainder of each day in a dark chamber (in the same greenhouse) through which a gentle current of air was drawn by means of a fan to prevent the temperature and humidity within the chamber from rising above that of the greenhouse. In spite of this precaution, the temperature within the chamber averaged from 2 to 3° F. higher than that outside, but the relative humidity remained approximately the same as outside.

The effect of the 6- and 10-hr. periods was expressed in an abnormally slow rate of growth and a partial assumption of a winter habit (Fig. 2). Curiously, the rate of growth was slower for the 10-hr. than for the 6-hr. plants. The plants were all infected at the same stage of growth, at the time of the emergence of the head, but, owing to the different rates of growth in the three lots of plants, it was not possible to inoculate them all at the same time. The plants subjected to full daylight headed and were inoculated with race 21 in the second week of May; those exposed to 6 and 10 hr. of daylight were inoculated about six weeks later, on June 19.

The infection results are given in Table IV. It is clear from an examination of Table IV that the plants in all three groups had acquired mature-plant resistance by the time they were infected, in spite of the radically different conditions of light to which they were subjected. This resistance was greatest in the plants grown in full daylight and least in those exposed to only six hours of daylight. It would seem, therefore, that while the effect of a reduced period of daylight is definitely in the direction of increased susceptibility, there is no real inhibition of the capacity of the plant to develop mature-plant resistance.

TABLE IV

THE EFFECT OF DIFFERENT PERIODS OF DAYLIGHT ON THE REACTION OF HOPE WHEAT TO RACE 21 OF *P. graminis Tritici*

The wheat was planted February 1

Pot	Reaction in terms of numerical values for infections					
	Leaves			Stems		
	6 hr. daylight	10 hr. daylight	Full daylight	6 hr. daylight	10 hr. daylight	Full daylight
1	11.5	4.0	—	14.5	8.0	11.0
2	6.6	9.0	2.0	13.7	10.0	—
3	12.0	10.0	2.0	14.1	11.7	14.5
4	13.3	9.5	—	17.5	7.4	5.3
5	10.7	8.3	6.0	14.6	8.4	9.7
6	7.2	7.3	2.0	14.6	9.1	7.0
7			—			4.7
8			4.2			11.0
9			8.5			6.2
Mean	10.2	8.0	4.1	14.8	9.1	8.7

The Effect of Temperature on the Reaction of Hope Wheat to Stem Rust

Simultaneously with the previously described experiment on the effect of different light periods on rust development, another experiment was conducted to test the effect of temperature on the reaction of Hope to race 21 of stem rust. The test plants were grown to maturity under three different conditions of temperature: (i) a constant low temperature (60° to 61° F.), (ii) a constant high temperature (75° to 80° F.), and (iii) a combination of these (10 hr. daily at the low and 14 hr. at the high temperature). The plants were grown in 6-in. pots, three plants per pot, and ten pots to each of the three different temperature groups. The plants were inoculated at the time of heading. As certain of the plants grown at constant high temperature headed slightly in advance of the others, these were inoculated about four days earlier than the rest. The infection results are summarized in Table V.

TABLE V

THE EFFECT OF TEMPERATURE ON THE REACTION OF HOPE WHEAT TO RACE 21 OF *P. graminis Tritici*

Pot	Reaction in terms of numerical values for infections					
	Leaves			Stems		
	61–61° F.	10 hr. 60–61° F. 14 hr. 75–80° F.	75–80° F.	60–61° F.	10 hr. 60–61° F. 14 hr. 75–80° F.	75–80° F.
1	—	4.5	4.0	11.0	—	—
2	2.0	—	—	—	9.0	—
3	2.0	—	5.0	14.5	—	15.0
4	—	2.0	—	5.3	10.5	—
5	6.0	6.0	8.0	9.7	8.9	13.3
6	2.0	—	4.7	7.0	10.2	11.8
7	—	4.0	7.5	4.7	8.5	14.0
8	4.2	—	5.3	11.0	10.0	13.3
9	8.5	—	4.7	6.2	9.0	9.0
10		8.0			4.3	
Mean	4.1	4.9	5.6	8.7	8.8	12.7

The tabular representation of the infection results shows that the plants in all three groups exhibited considerable resistance which was, however, least pronounced in the plants subjected to continuous high temperature. The reactions of the plants grown at low and at alternating low and high temperature were almost identical.

In a later test the breakdown of the resistance of Hope wheat at constant high temperatures was very pronounced. Plants grown and kept after inoculation at high temperatures (75° to 80° F.) developed infections averaging, in numerical values, 16.5 on the leaves and 15.8 on the stems, whereas plants grown and kept after inoculation at ordinary greenhouse temperatures

(55° to 80° F. daily) developed infections averaging 5.4 on the leaves and 7.5 on the stems. This represents a shift in reaction from resistance (infection types "1− 2=") at ordinary temperatures to susceptibility (infection type "3+") at high temperatures.

The Effect of Fertilizer on the Reaction of Hope Wheat to Stem Rust

An experiment designed to test the effect of fertilizer on the rust reaction of Hope wheat under conditions of reduced light intensity was conducted in the spring of 1939. This combination (the presence of fertilizer plus reduced light intensity) was thought conducive to the breakdown of mature-plant resistance. The plants were grown under one ply of cotton cloth that transmitted about 30% of light when tested with a Weston Photronic Exposure Meter in diffuse daylight. All plants were grown in 6-in. pots with three plants in each pot, one-half being grown in very light soil (one-third soil, two-thirds sand) which received no fertilizer, the other half in similar soil supplied monthly with a full nutrient solution (Shive's solution). All plants were inoculated with race 21 of *P. graminis Tritici* when the heads were beginning to emerge. Inoculation took place at the end of May and notes were taken on rust infection on June 12.

In this experiment there was very little difference in the reaction of the plants that received fertilizer and those that did not, despite the fact that the former were distinctly more succulent. The plants grown in non-fertilized soil were rather highly resistant; those grown in fertilized soil moderately resistant (Table VI). In no instance was there any breakdown in mature-plant resistance, a fact which suggests that this type of resistance is not readily influenced by the application of fertilizer.

TABLE VI

THE EFFECT OF FERTILIZER ON THE REACTION OF HOPE WHEAT TO RACE 21 OF *P. graminis Tritici*

Pot	Reaction in terms of numerical values for infections			
	Leaves		Stems	
	Fertilizer present	Fertilizer absent	Fertilizer present	Fertilizer absent
1	7.0	5.3	5.6	7.2
2	5.0	5.0	8.0	5.0
3	8.0	5.0	9.2	5.0
4	5.0	5.0	9.0	5.0
5	8.0	—	8.2	6.6
6	8.0	—	10.3	4.7
7	10.0	8.5	8.8	8.0
8	8.5	—	10.0	—
9	—	—	6.5	—
10	7.5	7.0	10.8	8.0
Mean	7.4	6.0	8.6	6.2

Discussion

Although mature-plant resistance occurs in many new wheat varieties derived from crosses with Hope or H 44, its nature is as yet little understood. Hart and Zaleski (5) have shown that this type of resistance finds its greatest expression under conditions of high light intensity while low intensity of light may result in its breakdown to a greater or less degree.

Although some of the experiments reported in this paper have confirmed the above-mentioned conclusions, it should be emphasized that the shading experiments thus far conducted have not consistently resulted in such a breakdown. The lack of consistent results in these experiments is puzzling. If the mature-plant resistance of Hope is fundamentally conditioned by light, it would be expected that drastic reduction in light intensity would always result in marked increase in susceptibility to rust. Such does not seem to be the case. Plants of Hope and other varieties with the same type of resistance have frequently been inoculated in the greenhouse during midwinter, when solar radiation is less than one-quarter of that of midsummer, and the amount of light received by the plants is frequently still further reduced by heavy coatings of frost on the roof of the greenhouse. Under such unfavourable light conditions it might be expected that these varieties would invariably show a certain degree of susceptibility, yet plants infected in midwinter often show rather high resistance.

In the two experiments in which shading resulted in increased susceptibility, there was a rather marked modification in the growth of the shaded plants. They were softer to the touch and noticeably less rigid than the plants grown in full light. Dry weight determinations showed that the moisture content of both stems and leaves was considerably higher in the shaded plants. It is likely that the increased succulence of the shaded plants is mainly a consequence of the low light intensity at which they were grown, although the somewhat higher humidity inside the cheesecloth cages may also have contributed. As the shading of the plants raised the humidity by only about 10% whereas it decreased the light intensity by 60 to 70%, it is likely that the greater share of the increased succulence and susceptibility of the shaded plants should be attributed to the low intensity of light under which they were grown. Possibly reduced light intensity is capable of increasing the susceptibility to rust only when other growing conditions are such as to lead to an increase in the moisture content or succulence of the plants.

As mentioned elsewhere in this paper, mature-plant resistance, although generally effective in North America, has been known to break down in other parts of the world, such as Peru, South America, and Kenya, East Africa. In view of the fact that the daily period of light is shorter in these equatorial regions than it is during the wheat growing season in Canada and the United States, it was thought possible that Hope plants grown in the greenhouse under short day-length conditions might be more susceptible to stem rust than plants grown under conditions of longer day-length. The experimental evidence obtained tends to support this idea, although even the plants grown under

conditions of only six hours daylight per day appear to possess a certain degree of resistance. The plants grown in 10 hr. of daylight and those grown in full daylight were, however, considerably more resistant. The reduction in the daily period of light had the effect of forcing the plants for a while into a semblance of winter habit. When, however, the plants finally emerged from this growth habit and came into head they appeared to have acquired a certain degree of mature-plant resistance in spite of the short day-length. Nevertheless the experimental results do suggest that plants grown under conditions of short day-length are less highly resistant than plants grown under longer daylight periods.

The reduction in light intensity required to shift the reaction of Hope wheat from resistance to moderate susceptibility is so great that it is difficult to imagine that low light intensity could be the main factor in causing breakdown in mature-plant resistance in nature. In view of the experiment on the influence of day-length on the rust reaction of Hope, it is equally difficult to explain the breakdown of mature-plant resistance in equatorial regions as merely the effect of a shorter period of daylight. The plants grown in 10 hr. of daylight—a period shorter than the day-length of tropical regions—were rather highly resistant. Where the light period was reduced to six hours per day there was, however, a partial breakdown of resistance. As far as can be judged from this experiment, therefore, a daylight period such as that prevailing in Peru or Kenya would, of itself, be insufficient to account for the reaction of Hope and similar varieties in these regions.

Three other factors were considered in this paper, namely, soil moisture, soil nutrient supply, and temperature. The experimental results indicate that increase of soil moisture and supply of soil nutrients increase the succulence of the plant and thereby its susceptibility. There is some evidence that the mature-plant resistance of Hope wheat can be broken down at high temperatures. The infection results tend to confirm the idea that progressively higher temperatures lead to a progressive increase in susceptibility (6, 10, 14). This increase, however, reaches its limits at abnormally high temperatures as shown by Johnson and Newton (8), and there must therefore be some temperature point at which susceptibility no longer increases but begins to diminish. The experiments on temperature reported in the present paper suggest that the susceptibility of Hope increases with rising temperature contrary to observations recorded by Hart and Zaleski (5). As they reported a temperature variation from 26° to 33° C. (79° to 91° F.), it is possible that, in their experiment, the temperature had reached a point beyond the maximum at which susceptibility continues to increase.

Despite such attempts as have been made to elucidate the nature of mature-plant resistance, it must be admitted that the problem is still far from being solved and, as yet, not even the influence of environmental conditions on the expression of resistance is fully understood. There can be little doubt that conditions of light do influence rust reaction, but so also do conditions of temperature, moisture, and soil nutrients. The experimental results secured

in the work here described indicate that deficiency of light can increase susceptibility in Hope only in conjunction with other factors which influence plant development in the direction of increased succulence. It would appear that there is an inherent tendency in Hope to acquire resistance to rust as the tissues become mature, and that very subnormal light conditions, moderately high temperatures, and other factors exert a repressive influence on this process.

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INTERSPECIFIC AND INTERGENERIC CROSSES WITH *HORDEUM*[1]

By F. L. Quincke[2]

Abstract

Crosses are described between *Hordeum jubatum* (28 chrom.) × *Hordeum vulgare* (14 chrom.), *H. nodosum* (42 chrom.) × *H. vulgare*, *H. vulgare* × *Secale cereale* (14 chrom.), *H. jubatum* × *S. cereale*. In the interspecific crosses viable seeds were obtained which produced healthy and vigorous plants. The seeds of the intergeneric crosses did not germinate.

The enormous soil losses of the Western Plains due to wind and water erosion necessitates the production of winter hardy cereals which will cover the land during the critical period of the late winter and early spring. A few crosses have been made with the hope of developing a winter hardy barley for the Northwestern Plains.

Hordeum jubatum × Hordeum vulgare hex. (var. Friedrichswerther)

When pollen of the *H. vulgare* was transferred to the stigma of *H. jubatum* approximately 70% of the florets set seed. The F_1 seeds were slightly shrunken as compared with the selfed seed of *H. jubatum* and only 9% germinated. The plants developed somewhat slowly at first. After four weeks they produced as much top growth as the female parent. At heading stage the F_1 exceeded the female parent considerably in size and amount of foliage. The F_1 plants retained the perennial habit of the female parent but differed from the latter in having long auricles, short pubescence, well developed lateral florets, larger florets and shorter awns.

Hordeum jubatum × Hordeum vulgare dist. (var. Rex)

In this cross 78% of the pollinated florets set seed. Of the seed obtained, 54% produced strong and vigorous plants, of a height about twice that of the female parent. The pubescence of the female parent was lacking in the F_1 plants.

Hordeum nodosum × Hordeum vulgare dist. (var. Hanna)

Forty-three per cent of the florets pollinated with pollen of the variety Hanna set seed. Twenty-eight per cent of the seeds were viable. The F_1 plants showed considerable vigour as compared with the female parent. They also lacked pubescence.

[1] *Manuscript received February 6, 1940.*
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*Hordeum vulgare (var. Friedrichswerther) × Secale cereale (var. Crown)**

When pollen of *S. cereale* var. Crown was placed on the stigma of *H. vulgare* var. Friedrichswerther approximately 90% of the florets set seed. The matured seeds were very shrunken. When dissected, they showed no traces of embryo or endosperm. A detailed investigation after pollination revealed that while fertilization of the embryo and endosperm nucleus took place, the development of both soon ceased. Eight days after fertilization both endosperm and embryo had disintegrated.

Hordeum jubatum × Secale cereale (var. Crown)

Ninety-five per cent of the florets pollinated set seed. The seed was very shrunken and failed to germinate. When dissected it showed a well developed embryo sac without any traces of the embryo or endosperm.

* *The writer is much indebted to Dr. W. P. Thompson for his cytological study of the cross H. vulgare × S. cereale which was discussed by the latter in his paper on "The Frequency of Fertilization and the Nature of Embryo and Endosperm Development in Intergeneric Crosses in Cereals" given before the Genetic Congress in Edinburgh, 1939.*

NITRIFICATION UNDER AND AFTER ALFALFA, BROME, TIMOTHY, AND WESTERN RYE GRASS

V. BIOLOGICAL ASSAYS OF HAY CROP RESIDUES[1]

By R. Newton[2] and R. S. Young[3]

Abstract

As part of a study of crop sequence effects, the possible toxicity of fermentation products of crop residues was investigated. Adding residues to soil samples caused stimulation rather than inhibition of nitrification rates in the laboratory. Extracts of the residues fermented for different periods were added to cultures of *Penicillium* and of wheat plants. Here both toxic and vitamin-like effects were noted, the latter being dominant except in extracts from residues at early stages of decomposition. Effects of both kinds were transitory, but probably significant, a conclusion supported by considerable specificity noted in influence on fungus flora of soil.

Introduction

Earlier papers in this series dealt with residual effects of alfalfa (*Medicago sativa* L.), brome (*Bromus inermis* Leyss.), timothy (*Phleum pratense* L.), and western rye grass (*Agropyron tenerum* Vasey), on wheat crops at Edmonton, Alberta, as indicated by nitrogen absorption of crops (6) and nitrate accumulation and numbers of soil micro-organisms (4); and attempted to explain these by the composition of the hay crop residues (7), and the oxidation-reduction potentials and carbon dioxide production of residues and soils (3). The investigations covered the eight seasons 1927–34. Quite early in their course it began to appear that differences in the composition of the roots and stubble of the four hay crops might not account wholly for the differences in their after-effects. Accordingly the writers decided to re-examine the contentious question as to whether toxic decomposition products of the residues influenced subsequent wheat crops, either directly or through the soil microflora.

The method of biological assay, that is, of determining the presence of an active principle by its effect on the growth or health of some organism, was applied to fresh and fermenting crop residues. The test organisms used in the assays were nitrifying bacteria (in unsterilized soil), pure cultures of a blue mould, *Penicillium cyaneo fulvum* Biourge*, and common wheat.

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* *Kindly identified for the authors by Dr. Charles Thom, Principal Mycologist, Bureau of Plant Industry, Washington, D.C.*

Crop Residues Used

Sods were dug in the spring of 1930, before new growth had begun, to a depth of 10 in. They were washed to remove the soil as thoroughly as possible, dried, and the roots and stubble carefully divided at the crown for separate analysis. For these biological assays, however, the roots and stubble, after grinding in a Wiley mill to pass a 1-mm. sieve, were combined again in equal parts, this being roughly the ratio of their occurrence in one-year-old sods—the only sods in the main experiment that had been analysed up to that time. The mixed residues thus prepared were stored in tightly closed cans for use as required during a period of about a year.

It was not possible to take sods in sufficient quantity from the main experimental block without interfering with the subsequent course of the experiment. Accordingly, sods were dug from other available blocks. Since the residues differed significantly in composition from those of the main experiment (7, Tables I and III), the corresponding information for those used in the biological assays is given now in Table I.

TABLE I

COMPOSITION OF ORGANIC MATTER IN MIXED RESIDUES USED IN BIOLOGICAL ASSAYS

Crop	Age of sod, yr.	Crude protein, %	Ether extract, %	Crude fibre, %	N-free extract, %
Alfalfa	5	18.5	2.2	40.4	38.9
Brome	6	17.2	2.4	32.0	48.4
Timothy	2	17.9	3.2	27.5	51.4
Western rye	2	15.1	3.1	35.0	46.8

Crop	Reserve* carbohydrates, %	Ratio carbohydrate† to protein	Ratio crude fibre to N-free extract
Alfalfa	16.9	4.3	1.04
Brome	21.6	4.7	.66
Timothy	19.9	4.4	.53
Western rye	19.1	5.4	.75

* *Reducing sugar as dextrose after 3 hr. hydrolysis of residues in 1% hydrochloric acid on boiling water bath.*

† *Carbohydrate taken as sum of crude fibre and nitrogen-free extract.*

The nitrogen levels in these residues are considerably higher than those found in the main experiment, and the difference in this respect between the alfalfa and the grasses is much less. The ratios of carbohydrates to protein are correspondingly lower. These were the characteristics of the residues found in the main experiment to be most closely related to observed sequence effects. "Reserve carbohydrates", the new quantity included in Table I, represent an attempt to assess the readily available energy values of the

residues. Spoehr concluded in his investigation of cacti (8) that hydrolysis of dried, ground tissue in 1% hydrochloric acid for three hours under a reflux condenser on a boiling water bath converted starch and other normal reserve materials to monosaccharides, without affecting the cellulose. This method was applied to hay crop residues. The quantities found will obviously be related to the previous history of the stands sampled, and it has been explained that the samples were taken from different blocks, not uniformly treated. The values must therefore be regarded as peculiar to these samples, and not as affording a representative comparison of the four crops. The same consideration applies, though in lesser degree, to all the data in Table I.

Nitrification in Soil in Presence of Crop Residues

The observation by many workers, referred to in Part I (6), that the addition of carbohydrates to soil, as in straw or grass residues, causes a temporary disappearance of nitrates by assimilation in the bodies of micro-organisms fermenting this material, as well as our interest in the possible occurrence of toxic decomposition products, led us to try first the effect of the residues in controlled nitrification experiments. The plan of the experiment was similar to that used by Wilson and Wilson (9) in comparing nitrification of corn and sorghum roots. Twelve sets of five flasks each, comprising a check containing 100 gm. of dry soil only, and four flasks in which 0.5 gm. of residues were incorporated in the soil, were incubated at 25° C., and nitrates determined periodically by the phenoldisulphonic acid method (2). The soil was fertile Edmonton loam, containing about 15% organic matter and 0.60% nitrogen; it was sifted to remove coarse fibre. The flasks were brought to optimum moisture content, taken as 50% of the water-holding capacity of soil + residue, with a solution of calcium nitrate of such concentration as to add nitrate nitrogen equivalent to 40 p.p.m. of dry soil. The calcium nitrate was added, not to accentuate the disappearance of nitrates, as Wilson and Wilson used it (their soil being much poorer initially), but to minimize the effect of variations, due to sampling errors, in the original nitrate content of the soil in different flasks. An extra set of flasks was set up and analysed at once to determine the initial concentrations.

The experiment was carried out a second time, using the residues in approximately equal parts by weight of nitrogen, with brome and timothy still at 0.5 gm. The initial concentration of nitrate nitrogen in the five flasks analysed at once ranged from 128 to 133 p.p.m.

The results of both experiments were essentially alike, and only the second set need be presented. It is clear from Fig. 1 that there was neither any inhibition by toxic decomposition products nor any disappearance of nitrates. On the contrary, all the residues stimulated slightly the production of nitrates.

It may be thought that 0.5 gm. residues in 100 gm. soil, which caused a substantial disappearance of nitrates before they began to accumulate again in the Dunkirk gravelly sandy loam used by Wilson and Wilson (9), was not

enough to influence greatly the course of nitrification in Edmonton loam, which is much richer in organic matter. It should be pointed out, however, that this proportion of residues is of the same order as that added in the top six inches by the sods of our four crops in the field, where they were followed by significantly different nitrification rates (6). On the other hand, the uniformly favourable conditions for nitrification provided in the laboratory experiments would not usually be realized in the field.

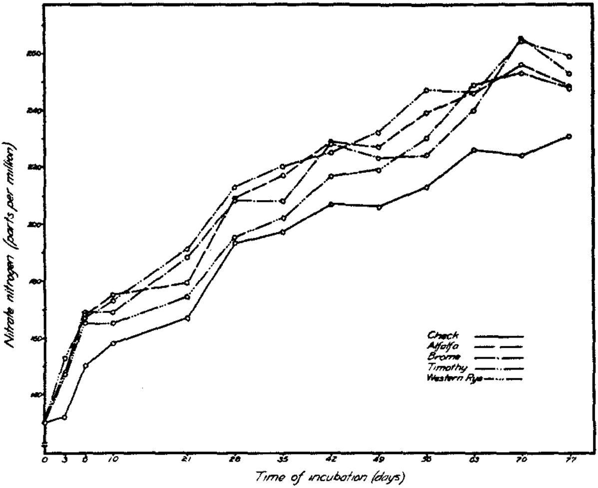

FIG. 1. *Rate of nitrification in Edmonton loam in presence of various crop residues. Series II, 1930.*

Preparation of Extracts of Fermenting Residues

For the assays with *Penicillium* and wheat plants, the residues were not used directly but in the form of extracts. Two series of experiments were carried out in which portions of the residues were fermented in a mixture with washed sand in Mason jars. In Series I they were mixed with sand in the proportion of 15% dry weight of the residues, a proportion roughly equal to that of organic matter in Edmonton loam. In Series II the same proportion of alfalfa residues was used, but the grass residues were added to their respective jars on the basis of equivalent quantities of organic matter, it having been discovered in the meantime that their ash content was abnormally high because of the impossibility of freeing the fibrous roots completely from

adhering soil particles. In both series of experiments the tops of the jars were left open for aeration, but in the first the one-quart jars were nearly filled by the quantities used, while in the second the two-quart jars substituted were only about half-filled, thus making it convenient to "cultivate" (stir up) weekly this synthetic "soil", as a precaution against the development of partially anaerobic conditions at the bottom. Five sets of jars were prepared for each experiment, representing fermentation periods of 0, 3, 6, 9, and 12 weeks. The mixture in each jar was made up to optimum moisture content, taken as 50% of the water-holding capacity, with distilled water to which had been added for each jar 10 cc. of a suspension of soil organisms made by shaking 100 gm. of Edmonton surface loam with 200 cc. of water. The jars were stored at 25° C., and weighed every two or three days for the purpose of making up to optimum moisture content.

At the end of the fermentation period, the contents of each jar were transferred to an eight-litre bottle and extracted with five parts by weight of distilled water to one part of the "soil", by shaking for 10 min. at 20° C. in a large shaking machine. The extracts were filtered and stored in Winchesters at 0° C. They were brought to a uniform nitrate content by adding calcium nitrate up to the concentration found in the one naturally richest in nitrate. The initial value (unfermented) in Series I was 2 to 3 p.p.m., but this dropped to less than 1 p.p.m. in three weeks and remained stationary thereafter, perhaps owing to the development of partially anaerobic conditions. In the better aerated Series II, nitrate increased steadily to about 60 p.p.m. at 12 weeks, and all extracts in this series were made up to this concentration. The portions used for the assays with *Penicillium* were sterilized by filtration through Pasteur-Chamberland candles.

Certain properties of these extracts which might be related to their activity were determined. The oxidation-reduction potentials did not vary significantly. Hydrogen ion concentrations fell chiefly in the range pH 7 to 8, with the higher values in the extracts of fermented, as compared with fresh, residues; there were no consistent differences between the extracts of the different residues. The remaining properties determined—total sugar, total soluble organic matter, lead acetate precipitate, and colour intensity—did show significant differences, and the data for Series II, the better aerated set of jars, are assembled in Table II.

Total sugar was determined as reducing sugar in an aliquot of 50 cc. after clarifying the extracts with lead acetate, deleading with disodium phosphate, and inverting with citric acid. Total soluble organic matter was determined by evaporating 10-cc. portions of the extracts to dryness and igniting. Relative colour intensity of the extracts after passing through a Pasteur-Chamberland filter was determined with a colorimeter, using the palest extract, that of alfalfa after 12 weeks' fermentation, as a standard. The precipitate obtained by adding 1 cc. of molar lead acetate solution to 25 cc. of extract in a test-tube, shaking, centrifuging, filtering with washing, drying *in vacuo* at 98° C., and weighing, gives another estimate of substances colouring the extracts. In the

TABLE II

PROPERTIES OF EXTRACTS OF FERMENTING CROP RESIDUES, SERIES II

Residue \ Period of ferment'n	None	3 wk.	6 wk.	9 wk.	12 wk.
Total sugar (mg. in 100 cc.)					
Alfalfa	17.2	15.0	6.6	6.6	0.0
Brome	38.6	4.0	13.8	6.6	15.0
Timothy	18.0	10.2	9.4	4.0	0.0
Western rye	59.8	23.6	6.0	18.0	21.0
Total soluble organic matter (mg. in 100 cc.)					
Alfalfa	215	57	73	73	74
Brome	119	146	32	134	106
Timothy	190	137	127	137	100
Western rye	154	57	89	110	68
Lead acetate precipitate (mg. in 100 cc.)					
Alfalfa	79	128	52	46	50
Brome	120	176	86	67	86
Timothy	113	180	99	84	115
Western rye	76	125	66	69	58
Relative colour intensity					
Alfalfa	1.5	3.3	1.7	1.0	1.0
Brome	3.2	5.5	3.2	1.9	3.1
Timothy	3.0	5.5	4.7	3.3	4.7
Western rye	1.5	3.2	1.7	1.5	1.1

Colour quality key: 1.0–1.1 pale yellow; 1.5–1.7 yellow; 1.9 deep yellow; 3.0–3.3 reddish-brown; 4.7–5.5 dark reddish-brown.

table, the analytical values are converted to the basis of 100 cc. extract for ease of comparison.

In sugar, western rye extract was highest and brome next. In all residues the sugar tended to disappear during fermentation, though not at a uniform rate. The same is roughly true of soluble organic matter; here alfalfa and timothy are highest initially. Total organic matter does not appear to be related to colour of extract, but lead acetate precipitate obviously is.

Assays with Penicillium

The blue mould *Penicillium* was selected because it is a rapid-growing organism forming a thick compact mat of mycelium which can be filtered and weighed readily. Moreover it is a typical soil-inhabiting fungus.

Three series of assays were made of the water extracts of fermenting residues. In Series I, the extracts came from the first lot of fermented mixtures, made during the early summer while the residues collected that spring were still quite fresh. Series II and III were made with extracts of the second lot, fermented after the dried, ground residues had been an additional seven months in storage. The growth of the fungus at 20° C. on Czapek's nutrient solution in 200-cc. Erlenmeyer flasks, to which extracts were added in graduated quantities, was measured by the dry weights of the mycelial mats produced. The flasks were liberally and uniformly seeded with freshly produced spores and allowed to incubate four days in Series I and II, and two days in Series III, before adding the extract, in order to avoid complicating the results by the possible effect of the extract on germination. The fungus was filtered off seven days later, experience having shown that this covered the period of maximum growth rate. The concentration of the Czapek solution was adjusted so that after the addition of the graduated quantities of extract to the flasks the final concentration of nutrients was standard in all flasks. The final volume of solution and extract was 60 cc. in all flasks, except in the first group (no fermentation) of Series I, in which the addition of 10, 20, and 30 cc. of extract brought it to 60, 70, and 80 cc. respectively. The results are given in Tables III, IV, and V.

TABLE III

MEAN WEIGHTS (MG.) OF TRIPLICATE* CULTURES OF *Penicillium* CONTAINING EXTRACTS OF FERMENTING CROP RESIDUES

Series I, 1930

Kind of residues	Volume of extract added, cc.	Length of fermentation of residues		
		None	6 wk.	12 wk.
Control	—	257	379	353
Alfalfa	10	475	530	354
	20	555	585	389
	30	626	555	381
Brome	10	464	514	364
	20	587	526	389
	30	658	538	383
Timothy	10	437	568	363
	20	584	489	383
	30	607	202	381
Western rye	10	431	524	375
	20	535	534	373
	30	668	617	365

* *Control values in Tables III, IV, and V are means of 9 replicates.*

For no fermentation time and 6 weeks:

Standard error = 83

Necessary difference between control and treatment means = 111 to 133†

Necessary difference between treatment means = 118 to 152†

† *Vary owing to accidental gaps in data.*

In the first series of assays, reported in Table III, only the unfermented and the 6- and 12-week extracts were used. It will be seen that extracts of unfermented residues and of residues fermented six weeks, except the highest concentration of timothy six-week extract, stimulated the growth of *Penicillium*. Similar experiments by Newton, Lehmann, and Clarke (5) in the same laboratory had demonstrated that phenolic substances ordinarily toxic to organisms exerted in high dilution a stimulatory effect on growth. A series of graduated dosages on the fungus *Helminthosporium sativum* produced a physiological curve of stimulation and inhibition. The same mechanism seems applicable to the results with the six-week timothy extract, but in the other cases the effect seems more like that of a vitamin, since there is no tendency to inhibition of growth with increasing concentration. It has already been mentioned that in the assays of unfermented residues reported in Table III, the volumes of culture solution were increased by the addition of the extracts, and this was suspected of causing part of the increased growth. This defect in technique did not occur in the rest of the work. All effects, whether of stimulation or inhibition, had practically disappeared in the extracts from residues fermented 12 weeks.

The second series of assays was made with extracts from the second batch of the fermented residues, which differed from the first batch mainly in the weekly stirring, or aeration, of the sand-residue mixtures in the jars. This,

TABLE IV

MEAN WEIGHTS (MG.) OF TRIPLICATE CULTURES OF *Penicillium* CONTAINING EXTRACTS OF FERMENTING CROP RESIDUES

Series II, 1931

Kind of residues	Volume of extract in 60 cc.	Length of fermentation of residues				
		None	3 wk.	6 wk.	9 wk.	12 wk.
Control	—	298	394	321	347	329
Alfalfa	10	364	362	375	322	298
	20	417	441	427	331	398
	30	437	412	445	344	364
Brome	10	398	50	408	366	388
	20	465	27	432	365	418
	30	463	2	443	395	437
Timothy	10	412	44	418	364	404
	20	470	7	347	390	427
	30	485	29	337	435	463
Western rye	10	357	191	339	374	512
	20	426	227	412	360	557
	30	450	311	385	378	525

Standard error = 31
Necessary difference between control and treatment means = 41
Necessary difference between treatment means = 50

of course, could not influence the character of the extract from the unfermented residues, yet it will be seen in Table IV that the vitamin-like effects were less marked. The dried, ground residues had been an additional seven months in storage, and it is known that vitamins may diminish under these conditions. The aeration may, however, have accelerated the rate of both appearance and disappearance of transitory substances, and perhaps accounts for the lack of any pronounced inhibition of growth by timothy extract at six weeks. Fortunately the three-week extracts were included in this series, and both brome and timothy showed marked toxicity at this stage, while growth in the presence of western rye extract was below that in the control and in the alfalfa. The vitamin-like effects, where they appeared, were not significantly different for the 20 and 30 cc. additions of extract. In the longer fermentation periods, such effects as persisted were practically all stimulatory in nature.

The third series of assays was done with portions of the same extracts used in Series II, but after they had been kept about a month in storage at 0° C. Certain additional refinements of technique were introduced, in the hope of reducing the variability of the results, notably the use of a single-spore culture of the test organism. The other main difference from the previous series was in the use of larger increments of extract, viz., 10, 30, and 50 cc. (as compared with 10, 20, and 30 cc.) with the object of surpassing definitely

TABLE V

MEAN WEIGHTS (MG.) OF TRIPLICATE CULTURES OF *Penicillium* CONTAINING EXTRACTS OF FERMENTING CROP RESIDUES

Series III, 1931

Kind of residues	Volume of extract in 60 cc.	Length of fermentation of residues				
		None	3 wk.	6 wk.	9 wk.	12 wk.
Control	—	320	391	403	407	382
Alfalfa	10	508	408	414	379	329
	30	583	584	503	405	404
	50	541	423	504	441	412
Brome	10	465	194	436	393	412
	30	548	181	526	437	430
	50	551	89	546	480	376
Timothy	10	531	159	439	392	395
	30	593	131	432	479	455
	50	553	12	330	513	379
Western rye	10	487	312	396	368	354
	30	571	205	474	459	430
	50	560	108	509	471	477

Standard error = 33
Necessary difference between control and treatment means = 43
Necessary difference between treatment means = 53

the maximum in case the vitamin-like effect were merely a stimulation by a toxic substance in low concentration. The results suggest, however, that in most cases this effect was not due to a toxic substance. The maximum stimulation appears to have been attained by the addition of 30 cc. of extract and, comparing treatment with control values, there was no inhibition by 50 cc. of extract. The toxic effect, however, which again showed plainly, though less extremely than in Series II, in the three-week extract of the grasses, increased with the volume of extract added. There was also an appreciable inhibition of growth by 50 cc. of timothy extract from the six-week fermentation period.

A singular feature of the control cultures, to which no plant extract was added, was the complete absence in Series III of the typical green colour of the spore mass with which the mycelial mat was covered. Apparently the particular single-spore strain used in this series of cultures was incapable of developing its normal pigment in the absence of the organic supplement represented by the extracts. This did not happen in Series I or II.

Assays with Wheat Plants

Wheat plants were used as test organisms in much the same way as *Penicillium*. The wheat was grown in glazed one-gallon crocks of pure sand half-saturated with Hoagland's nutrient solution and, like the extract jars, inoculated with a suspension of soil organisms. The kernels were seeded long enough before the fermenting residues were due to be extracted, to give them time to germinate and emerge, when they were thinned to six well-spaced, uniformly vigorous plants per crock. The extracts were then added periodically in quantity sufficient to maintain optimum moisture content in the sand culture which transpired least, the necessary additional moisture in the other cultures being made up with distilled water. Nutrients were added from time to time in accordance with ordinary practice. The moisture content of the controls was maintained with distilled water to which had been added nitrate in the same concentration as in the extracts. The plants were harvested six days after the extracts were exhausted.

Two series of assays were carried out, corresponding to the two lots of fermented residues. The results of both are given in Table VI. In Series I, comparisons must be limited to weights within each fermentation period, i.e., in a single column, since the wheat cultures were set up on five successive dates at intervals of three weeks, corresponding to the end of the fermentation periods of the various lots of residues. This probably made less difference to the *Penicillium* cultures grown under controlled conditions in the laboratory*, but did result in exposing the different lots of wheat plants to markedly different environments in the greenhouse as the season progressed from June to August, 1930. In Series II the wheat plants were all

* *Even here significant differences occur between control values, including those of Penicillium Series II and III, each of which was set up at five successive two-day intervals. Difficulty in duplicating conditions of inoculation on different days is a common experience of mycologists.*

TABLE VI

TOTAL DRY WEIGHT (GM.) OF SIX WHEAT PLANTS GROWN IN SAND CULTURE WATERED WITH EXTRACTS OF FERMENTING CROP RESIDUES

Kind of residues	Length of fermentation of residues				
	None	3 weeks	6 weeks	9 weeks	12 weeks
Series I					
Control	26.9	16.2	14.5	9.0	8.8
Alfalfa	11.7	11.6	3.1	8.3	8.6
Brome	32.0	22.4	4.4	5.7	10.3
Timothy	21.5	21.5	3.6	6.7	10.2
Western rye	26.8	24.7	7.4	7.4	8.8
Series II					
Control	20.4	24.6	26.1	25.3	23.2
Alfalfa	27.9	23.3	34.7	34.1	26.1
Brome	36.5	29.6	30.7	32.0	25.0
Timothy	37.3	26.7	33.2	32.6	21.4
Western rye	31.6	28.3	39.1	31.7	24.1

grown at one time, the residues having been set to ferment at three-week intervals so that all completed their respective periods approximately together. This wheat series was grown during the period January to March, 1931, when it was possible to control greenhouse conditions more satisfactorily.

In Series I it was surprising to find the extracts of unfermented and three-week alfalfa exercising a depressive influence on growth, since alfalfa extract had stimulated the growth of *Penicillium* at least as much as had that of the grasses, and unlike the grasses had in no case shown toxicity to that organism. In a biological study of the decomposing sods in the field (4) it had been observed that alfalfa sod favoured the development of fungi, especially *Mucor*, to a much greater extent than did the grasses. This was suspected to be the possible cause of the unthriftiness in the wheat grown in the sand cultures. Water suspensions from the sand cultures plated out did indeed reveal a predominance of large *Mucor* colonies in those treated with alfalfa extracts. The repressive effect of the alfalfa was possibly an indirect one, exerted through its stimulation of micro-organisms which competed with or injured the roots of the wheat. Thus the prime object of the technique developed for assaying these residues, namely, to separate the effects of chemical and biological factors, was scarcely fulfilled by the wheat cultures, and from this point of view they were less successful than the *Penicillium* cultures. The figures suggest that all the extracts from residues fermented six weeks may have slightly repressed growth, but the error of the experiment is too great to permit conclusions. Certainly there is no evidence of any effect at 9 and 12 weeks.

In Series II, done with extracts of residues fermented under better conditions of aeration, there is no evidence of growth inhibition, but the unfermented and three-week alfalfa stimulated the growth of wheat less than did the other extracts, again possibly owing to the greater profusion of certain fungi in the alfalfa cultures. At 6 weeks and 9 weeks, all the extracts stimulated the growth of wheat, but there was no effect at 12 weeks.

The greater weights of the plants in Series II is partly due to the greater age of the plants at harvesting. Because of the lower rate of transpiration during the winter, they were in full flower before the supply of extracts was exhausted, whereas in Series I they were barely heading when harvested. The possibility of toxic decomposition products affecting the results with the six-week extract in Series I cannot, however, be ruled out, in view of the observation mentioned earlier, that the conditions of fermentation had inhibited nitrification.

The nitrogen content of tops and roots separately was determined in the wheat plants of both series. The data are omitted to save space, but it may be said that the chief trend discernible was towards a higher percentage in the heavier, thriftier plants.

The plating of water suspensions of the sand cultures was carried out more completely in Series II, in view of observations in Series I referred to above. Three borings were taken from each crock, just prior to harvesting. For fungi, dilutions of 1 : 1000 were used; for bacteria, 1 : 1,000,000 and 1 : 10,000,000. Media used were No. 4 and No. 18, Fred and Waksman (1) for bacteria and fungi respectively. Plates were poured in quadruplicate. The counts are given in Table VII. The wide variations in numbers do not appear to be related to the yields of wheat.

It will be remembered that all sand cultures, both those producing the residue extracts and those in which the wheat was grown, had been inoculated with a suspension of Edmonton surface loam in order to promote normal microbial balance. No wide differences were found in the kinds of bacteria on the plates, and no differences were apparent in the size of bacterial colonies from the various sand cultures. The fungi, on the other hand, varied widely in kinds and size of colonies. The control and alfalfa plates from the crocks receiving extracts of unfermented residues had chiefly large *Mucor* colonies. Brome and timothy plates had a mixed flora including a few large colonies of *Mucor*. Western rye plates had a large number of *Penicillium* colonies, with very few other forms present.

The three-week plates showed a mixed fungus flora, including *Mucor*, *Penicillium*, *Aspergillus*, and *Fusarium*. The six-week plates gave a mixed flora in the control and western rye, but scarcely any *Mucor* colonies in alfalfa, brome, or timothy. The 9- and 12-week plates likewise gave rise to a mixed flora. On the whole the plates from the unfermented residues, the 9- and 12-week fermentation periods all showed about equal vigour of fungus growth, as judged by size of colonies, and more than shown by the three- and six-week periods. The fungi on the alfalfa and brome plates in the six-

TABLE VII

NUMBERS OF BACTERIA AND FUNGI IN SAND CULTURES OF WHEAT PLANTS WATERED WITH EXTRACTS OF FERMENTING CROP RESIDUES

Series II, 1931

Residue	Fungi	Bacteria
	Thousands per gm. dry sand	*Millions per gm. dry sand*
No fermentation		
Control	13.1	18.0
Alfalfa	15.6	37.5
Brome	22.7	61.6
Timothy	7.8	2.3
Western rye	113.5	60.2
3 weeks' fermentation		
Control	30.8	34.1
Alfalfa	6.6	12.4
Brome	24.2	36.6
Timothy	23.0	23.7
Western rye	12.9	27.4
6 weeks' fermentation		
Control	28.8	41.6
Alfalfa	15.9	49.9
Brome	3.9	41.8
Timothy	12.8	21.9
Western rye	26.1	59.2
9 weeks' fermentation		
Control	5.6	28.1
Alfalfa	5.3	41.2
Brome	21.1	24.8
Timothy	10.0	40.7
Western rye	15.7	55.0
12 weeks' fermentation		
Control	8.3	28.6
Alfalfa	7.5	21.8
Brome	13.0	39.0
Timothy	5.3	49.5
Western rye	7.0	52.1

week group were particularly lacking in vigour. This specificity, both as t kind and vigour of colonies, is additional evidence of the biological effects c the extracts.

At the time of taking samples of the sand cultures for plate counts, sampl were also secured for the determination of pH. This ranged from 7.2 to 8. Cultures watered with fresh and three-week alfalfa extracts were a little low than the others, but all were within the range of healthy soil.

Discussion

While current soil theory for the most part rejects toxic decomposition products as factors in crop sequence effects in normally aerated soils, such an extreme view seems not wholly tenable in the light both of our own results and of certain other cases reported in the literature. We did not indeed demonstrate with certainty any toxicity in the wheat plant cultures. The extracts used in Series I were from poorly aerated jars, possibly comparable to "sour" soils. The effect of the alfalfa extract was thought to be indirect. But the effect of the extracts, even from well aerated jars, on the growth of *Penicillium* was undoubted, and all the extracts showed characteristic effects on the fungus flora of the sand cultures. This indicates the likelihood that decomposition products of crop residues influence soil micro-organisms, whose activities influence in turn the productivity of the soil and may also affect the growth of plants by parasitism.

Both the toxic and vitamin-like effects observed deserve equal consideration, since in practice it does not matter whether the differences it is desired to explain in yield and composition of wheat following various other crops on similar soil arise in particular cases from stimulation or inhibition of soil microbial activity. Further, it need not be assumed that only organic decomposition products are active in this respect. The vitamin-like effect may be due to "minor" or "trace" elements absent from our culture solutions and present in the extracts. Again, some species of plants accumulate toxic elements (e.g., selenium in *Astragalus*) which, on decomposition of the residues, must be liberated in the soil.

While due caution must be observed in applying laboratory results to field conditions, there is no doubt that crop residues do cause differences in the microbiological activity and yielding power of soil, and we have only to consider whether these experiments suggest a reasonable explanation. There is no need to discount the importance of other factors, such as differences in the C : N ratio of the residues, but our results do suggest that the biological action of substances leaching out of crop residues in the soil also play a part in modifying its productivity.

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AGRICULTURAL METEOROLOGY: A STATISTICAL STUDY OF CONSERVATION OF PRECIPITATION BY SUMMER FALLOWED SOIL TANKS AT SWIFT CURRENT, SASKATCHEWAN[1]

By J. W. Hopkins[2]

Abstract

Changes in the moisture content of summer fallowed soil in tanks 15 in. in diameter and 5 ft. deep set in pits in the centre of field plots at the Dominion Experimental Station, Swift Current, Saskatchewan, which have been recorded at fairly frequent intervals during the summer months since 1922, provided two series of data, comprising observations in May and June and those in July and August. These have been analysed statistically in order to relate conservation to precipitation after making allowance for other variable factors. Five-factor regression equations, fitted by the method of Least Squares, gave multiple correlation coefficients of 0.95 and 0.96 between the computed and observed conservation in the two series. It is inferred from these equations that under average conditions a one-day rain to the amount of 0.36 in. in May-June and 0.46 in. in July-August would be required just to offset the subsequent evaporation during a 10-day period. Increased penetration and retention of the heavier rainfalls is very definitely indicated, and it is calculated that whereas on the average 66% of a one-day rain of 1 in. in May-June would still be conserved at the end of 10 days, only 30% would be retained if the same amount was received in five daily showers each of 0.20 in. The intensity as well as the total amount of precipitation is thus emphasized as a factor of prime importance in its relation to soil moisture under semi-arid conditions.

Introduction

In regions of limited rainfall, the conservation by the soil of such amounts of precipitation as are received is a factor of the first importance in the conduct of agricultural operations. This is the case in the Prairie Provinces of Canada, where an appreciable proportion (according to Taggart (8) about eight million acres in Saskatchewan alone) of the cultivated land is summer fallowed each year in order to increase the supply of moisture available for the succeeding season's crop.

The amount of moisture thus accumulated is subject to considerable fluctuation, for the summer precipitation at any specified point in this region varies widely from year to year not only in total amount, but also in its mode of occurrence. Even in favourable seasons, a considerable aggregate is received in the form of daily amounts of 0.30 in. or less (4), whilst in months of below-average rain, the entire amount recorded may fall in this way. As a certain amount of the moisture received at any time as rain does not penetrate deeply into the soil, but is retained by the surface layer and subsequently evaporated directly into the atmosphere, it can readily be foreseen that the efficiency of conservation by the soil of different intensities of rain

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[2] *Statistician, National Research Laboratories, Ottawa.*

will not be the same, and that three separate showers of 0.25 in. for example, cannot be assumed to be equivalent in effect to one fall of 0.75 in. Thus it was the opinion of Barnes (1) that under western Canadian conditions the moisture from showers of 0.25 in. or less is generally lost within a day or two by evaporation. Daniel and Harper (2) adopted 0.50 in. per day as the lower limit of effective rainfall in Oklahoma, and a figure of 0.50 in. was also arrived at by McLaughlin, Blaney, and Taylor (7) in considering the disposition of winter rainfall in the Santa Ana river area of California. These last authors further observe that "every rainfall period must be studied separately in order to arrive at the penetration for the season, since the same seasonal precipitation gives entirely different amounts of penetration under different conditions of intensity and distribution."

As far as the writer knows, the actual penetration into the soil of individual rainfalls has not been studied quantitatively in any great detail in Canada. The technical difficulty of obtaining sufficiently accurate data for this purpose by the usual soil-boring procedures is considerable, if not insurmountable; whilst the exceedingly imperfect correlation between the rate of loss of water vapour from a body of soil, and that observed from instruments such as atmometers or from tanks or pans presenting a free-water surface, has been emphasized by several writers, of whom Thornthwaite and Holzman (9) may perhaps be noted as the most recent. It seemed therefore that advantage should be taken of the information relevant to this subject accruing from the operation of the deep, water-tight soil tanks at the Soil Research Laboratory, Dominion Experimental Station, Swift Current, Saskatchewan. This information is probably unique in that the tanks have been under continuous observation since the year 1922. The following sections of this paper accordingly describe the results of a statistical examination of the accumulated records, which were made available to the author through the co-operation of Dr. J. L. Doughty of the Soil Research Laboratory.

Observational Data

The construction, location, and management of the tanks has been described by the late S. Barnes (1). It will therefore suffice to mention here that they are made of galvanized iron, and are circular in cross-section, 15 in. in diameter and 5 ft. deep. The soil with which they are filled is said to have been uniform and well mixed, and to have been placed in the tanks in layers corresponding to the natural soil horizons. The tanks are set in pits, so that the top of the soil in each tank is approximately at ground level. A regular rotation is imposed, and in any particular season some of the tanks grow crops of wheat, grasses, etc., whilst others are summer fallowed. In the present communication, attention is confined to the latter.

For these, the observations consisted of the weights, determined at more or less regular intervals during the summer months, of the replicate tanks (usually four or six in number) fallowed in each of the years 1922 to 1938 (excluding 1923), together with daily records of precipitation secured from a

TABLE I

CONSERVATION OF PRECIPITATION BY SUMMER FALLOWED SOIL TANKS, SWIFT CURRENT, 1922–1938

May and June				July and August			
Period	No. of days	Precipitation, in.	Conservation, in.	Period	No. of days	Precipitation, in.	Conservation, in.
1 June to 15 June/22	14	0.37	−0.31	15 June to 19 July/22	34	5.13	+2.38
16 May 30 May/24	14	.89	+ .27	19 July 1 Aug./22	13	.20	− .38
30 May 6 June/24	7	.15	− .09	1 Aug. 12 Aug./22	11	1.15	+ .55
6 June 23 June/24	17	1.85	+ .71	12 Aug. 21 Aug./22	9	1.10	+ .33
23 June 4 July/24	11	.51	− .09	4 July 12 July/24	8	.67	+ .14
12 May 26 May/25	14	.28	− .14	12 July 18 July/24	6	.21	+ .11
26 May 9 June/25	14	1.21	+ .50	18 July 24 July/24	6	.37	.00
9 June 15 June/25	6	.59	+ .06	24 July 1 Aug./24	8	.81	+ .09
15 June 23 June/25	8	.00	− .19	1 Aug. 6 Aug./24	5	1.13	+ .67
23 June 29 June/25	6	.01	+ .11	6 Aug. 11 Aug./24	5	.73	+ .50
12 May 14 June/26	33	1.97	+ .22	11 Aug. 18 Aug./24	7	.15	− .20
14 June 2 July/26	18	1.67	+ .30	18 Aug. 25 Aug./24	7	.27	+ .16
16 May 14 June/27	29	4.75	+3.75	29 June 6 July/25	7	.18	− .24
14 June 25 June/27	11	.45	− .08	6 July 13 July/25	7	.66	+ .09
10 May 1 June/28	22	.52	− .20	13 July 25 July/25	12	.10	− .16
1 June 9 June/28	8	.42	+ .22	25 July 31 July/25	6	.64	+ .36
9 June 26 June/28	17	3.33	+2.40	2 July 9 July/26	7	.47	+ .03
26 June 5 July/28	9	1.44	+1.08	9 July 16 July/26	7	1.76	+ .97
7 May 20 May/29	13	.30	+ .24	16 July 21 July/26	5	.00	− .24
20 May 4 June/29	15	1.52	+1.02	21 July 28 July/26	7	.00	− .14
4 June 10 June/29	6	.00	− .09	28 July 2 Aug./26	5	.00	− .02
10 June 21 June/29	11	2.18	+1.58	2 Aug. 23 Aug./26	21	2.77	+1.44
21 June 28 June/29	7	.40	− .19	25 June 8 July/27	13	1.92	+1.07
5 May 13 May/30	8	.18	+ .05	8 July 18 July/27	10	.60	+ .03
13 May 20 May/30	7	.00	− .24	18 July 25 July/27	7	.35	− .14
20 May 27 May/30	7	.15	+ .09	25 July 1 Aug./27	7	.22	− .03
27 May 2 June/30	6	.00	+ .11	1 Aug. 8 Aug./27	7	.01	− .11
2 June 10 June/30	8	.29	− .08	8 Aug. 16 Aug./27	8	1.67	+1.08
10 June 17 June/30	7	1.52	+1.35	16 Aug. 22 Aug./27	6	.43	− .05
17 June 24 June/30	7	.04	− .08	22 Aug. 31 Aug./27	9	.71	− .03
24 June 3 July/30	9	2.04	+1.49	5 July 16 July/28	11	.19	− .52
16 May 28 May/31	12	.50	+ .28	16 July 23 July/28	7	.50	− .08
28 May 4 June/31	7	.02	− .16	23 July 30 July/28	7	.08	+ .11
4 June 11 June/31	7	.02	.00	30 July 7 Aug./28	8	1.06	+ .55
11 June 19 June/31	8	.27	− .02	7 Aug. 13 Aug./28	6	.00	− .17
19 June 25 June/31	6	.14	.00	13 Aug. 20 Aug./28	7	.14	− .11
25 June 2 July/31	7	1.24	+ .60	28 June 9 July/29	11	.53	+ .02
9 May 25 May/32	16	.55	+ .06	9 July 16 July/29	7	.77	+ .50
25 May 1 June/32	7	.21	+ .09	16 July 23 July/29	7	.00	− .42
1 June 15 June/32	14	1.92	+ .77	23 July 29 July/29	6	.00	− .14
				29 July 6 Aug./30	8	.00	− .02
15 June 22 June/32	7	.00	+ .03	3 July 8 July/30	5	.13	− .02
22 June 29 June/32	7	.39	+ .05	8 July 17 July/30	9	.02	− .56
11 May 31 May/33	20	1.26	+ .56	17 July 22 July/30	5	.27	− .02
31 May 8 June/33	8	.28	− .02	22 July 29 July/30	7	.17	− .03
8 June 15 June/33	7	.21	− .06	2 July 9 July/31	7	.41	+ .08
23 May 14 June/34	22	2.70	+1.69	9 July 16 July/31	7	.26	− .19
14 June 21 June/34	7	1.01	+ .06	16 July 22 July/31	6	.07	− .38
21 June 3 July/34	12	1.00	+ .16	22 July 12 Aug./31	21	1.96	+1.24
16 May 28 May/35	12	.33	+ .09	29 June 11 July/32	12	3.21	+2.65
28 May 6 June/35	9	.15	.00	11 July 20 July/32	9	.62	+ .05
6 June 18 June/35	12	.86	+ .33	20 July 27 July/32	7	.37	− .35
18 June 26 June/35	8	1.36	+ .78	27 July 3 Aug./32	7	.62	+ .39
26 June 3 July/35	7	1.06	+ .47	15 June 17 July/33	32	2.48	+ .64
3 June 23 June/36	20	1.71	+ .75	3 July 11 July/34	8	.16	− .20

TABLE I—*Concluded*

CONSERVATION OF PRECIPITATION BY SUMMER FALLOWED SOIL TANKS, SWIFT CURRENT, 1922–1938—*Concluded*

May and June				July and August			
Period	No. of days	Precipitation, in.	Conservation, in.	Period	No. of days	Precipitation, in.	Conservation, in.
23 June 6 July/36	13	.38	− .19	11 July 19 July/34	8	.15	+ .02
19 May 2 June/37	14	.71	+ .52	19 July 26 July/34	7	.87	− .42
2 June 10 June/37	8	.12	− .13	3 July 10 July/35	7	1.12	+ .41
10 June 16 June/37	6	.17	− .14	10 July 17 July/35	7	.00	− .31
16 June 25 June/37	9	.34	− .22	17 July 24 July/35	7	.56	+ .03
9 May 26 May/38	17	.36	.00	6 July 14 July/36	8	.20	− .14
26 May 1 June/38	6	.23	− .46	14 July 22 July/36	8	.35	− .05
1 June 7 June/38	6	.14	− .14	25 June 7 July/37	12	.05	− .25
7 June 14 June/38	7	.97	+ .55	7 July 21 July/37	14	.98	+ .49
14 June 22 June/38	8	.59	+ .05	21 July 11 Aug./37	21	.55	− .27
22 June 28 June/38	6	.18	− .19	28 June 5 July/38	7	.08	− .27
				5 July 13 July/38	8	.19	− .17
				13 July 19 July/38	6	.04	+ .06
				19 July 26 July/38	7	.37	− .08
				26 July 4 Aug./38	9	.17	− .14
Average	10.9	.78	+ .31	Average	9.0	.63	+ .15

rain-gauge adjacent to the tanks. Given the diameter of the tanks (15 in.), and the fact that 1 lb. of water at 15° C. occupies a volume of 27.73 cu. in., it follows that each change in weight of 1 lb. per tank represents a gain or loss of moisture equivalent to $27.73 \div \pi(7.5)^2$ or 0.157 in. in depth. By means of this relation the changes in weight over the various periods of record have been expressed in terms of inches of moisture, and are shown in Table I together with the corresponding totals of precipitation.

Results

Regression Analysis

From a priori considerations it may be inferred that the efficiency of conservation should show progressive changes correlated with the seasonal trends of soil and air temperature and wind velocity, which in a continental climate are quite pronounced. The available data are not particularly well adapted to the investigation of this point in detail, but are sufficiently numerous to permit of subdivision into those recorded during the first half of the season, namely May and June, and those falling in July and August. This is at once indicative of some seasonal effect, for although the average number of days between weighings (and hence the exposure to evaporation), shown at the foot of Table I, was greater in May-June than in July-August, the ratio of mean conservation to mean precipitation, at 0.31 : 0.78 in., or 40%, is more favourable in the former than in the latter period, for which it is 0.15 : 0.63, or 24%.

Fig. 1 shows the relation between the total precipitation and conservation observed in the individual periods. The seasonal shift is manifested in the tendency of the solid points representing the observations during July-August to lie below those for May-June. It may also be noted that the maximum total precipitation recorded in any period during which there was no net conservation of moisture is 0.87 in. for July-August, as compared to 0.52 in. for May-June.

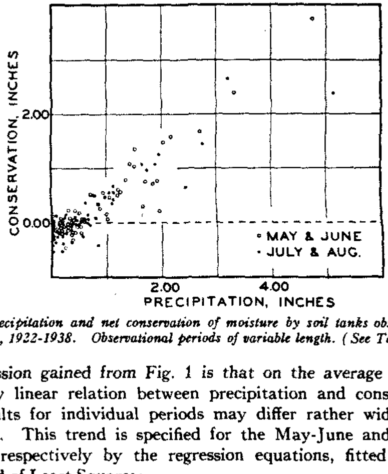

FIG. 1. *Precipitation and net conservation of moisture by soil tanks observed at Swift Current, Sask., 1922-1938. Observational periods of variable length. (See Table 1).*

The impression gained from Fig. 1 is that on the average there was an approximately linear relation between precipitation and conservation, but that the results for individual periods may differ rather widely from the general trend. This trend is specified for the May-June and July-August observations respectively by the regression equations, fitted to the data by the method of Least Squares:

$$c = -0.25 + 0.72\,p$$

and

$$c = -0.24 + 0.61\,p \qquad \text{(I)}$$

in which c denotes conservation, expressed as inches of depth, and p precipitation, likewise in inches.

These, however, can be regarded only as first approximations, their most obvious defect being that they do not take into account the differences in duration of the individual observation periods, and hence in the amount of evaporation to be anticipated. Inclusion of this factor significantly improved the regression equations, which now became as a second approximation:

$$c = -0.03 + 0.84\,p - 0.029\,d \quad \text{(May-June)}$$

and

$$c = -0.03 + 0.76\,p - 0.039\,d \quad \text{(July-August)} \qquad \text{(II)}$$

d being the interval between weighings in days.

Under similar atmospheric conditions, evaporation will be greater when the upper layers of soil are moist than when they are dry. The amount of evaporation during some of the observational periods may therefore have been influenced by precipitation occurring before, as well as during, the period in question. The total precipitation, as inches of depth (a) recorded during the seven days antecedent to each observational period was accordingly introduced into the two regression equations as an additional variable. In both cases negative regression coefficients resulted, indicating that in general above-average antecedent precipitation was associated with increased subsequent evaporation. However, only the coefficient for May-June can be regarded as statistically significant, leading to the conclusion that the observations in the latter part of the season were not subject to any considerable variation from this cause.

After the introduction of the quantity a the regression equations were:

$$c = +0.03 - 0.13a + 0.84\,p - 0.030\,d \quad \text{(May-June)}$$

and

$$c = +0.01 - 0.05a + 0.76\,p - 0.035\,d \quad \text{(July-August).} \qquad \text{(III)}$$

The effect of precipitation in moistening the upper soil may clearly be operative during observational periods as well as preceding them. It might perhaps be expected therefore that, other things being equal, the total loss of moisture recorded would be greater when rain occurred early in an observational period than when a similar amount fell towards the end, the first days being dry. In order to investigate this possibility quantitatively, a time factor was introduced into the regression function, consisting of the sum of products (t) of the daily rainfalls recorded multiplied by the ordinal numbers (reckoned from the end of each period) of the days on which they occurred. This was found to account for a significant fraction of the residual variance of conservation in both May-June and July-August. The Least Squares solution for the regression equations now took the following form:

$$c = +0.09 - 0.12\,a + 0.74\,p - 0.034\,d + 0.0091\,t \quad \text{(May-June)}$$

and

$$c = -0.09 - 0.05\,a + 0.84\,p - 0.026\,d - 0.0074\,t \quad \text{(July-August).} \quad \text{(IV)}$$

Although both of the additional regression coefficients are statistically significant, only one of them is negative in sign as anticipated. In scrutinizing the data for explanations of this discrepancy between the two series of observations, it was noted that several exceptionally heavy rains in May and June had chanced to occur early in the observational periods in which they fell. Attention was thus directed to the possibility that the relation between precipitation and conservation was not entirely linear, and that an increased effectiveness of these heavy rains was being confounded with the time factor. This point was investigated by expanding the regression function to include a quadratic term in precipitation, namely the sum of the squares of the daily amounts of rain recorded in each period. The sum of the squares, rather than the square of the sum, was used in order to discriminate between heavy

daily rains and similar totals received in smaller amounts over several days. Both series of observations yielded quadratic regression coefficients significant beyond the 1% point and positive in sign, indicating a proportionately greater conservation of the more intense rains. In view of this result, the cubic term in precipitation was also fitted, but proved to be statistically negligible. After the inclusion of the quadratic term, the regression equations were:

$$c = +0.08 - 0.12\,a + 0.34\,p + 0.42\,p^2 - 0.024\,d + 0.0120\,t \quad \text{(May-June)}$$

and

$$c = -0.07 - 0.03\,a + 0.52\,p + 0.33\,p^2 - 0.022\,d - 0.0000\,t \quad \text{(July-August).} \qquad \text{(V)}$$

The time factor within periods, t, is now reduced to insignificance in the July-August series, but its coefficient is still positive in the May-June equation, implying a greater conservation (temporarily at least) of precipitation received in the earlier part of the observational periods. No ready explanation of this tendency presents itself, except possibly the reduction in both soil and air temperatures following rainstorms, which might be expected to be more pronounced, and to persist longer, in the first half of the season. In the absence of soil temperature records, this point cannot be explicitly resolved.

The effect of variations in air temperature was however examined by the inclusion of this factor in the observational series. It was judged that evaporation would be affected, if at all, to a much greater degree by the daily maximum than by the mean air temperature, and the former quantity was accordingly the one employed. Even so, the resulting improvement in the goodness of fit of both the May-June and July-August regression equations was statistically quite insignificant. It must be concluded, therefore, that within series the recorded variations in daily maximum air temperature, apart from those associated with the incidence of precipitation, were without demonstrable effect on the amounts of moisture conserved by the soil tanks.

Adequacy of Regression Function

After the fitting of Equations (V), an analysis of the variance of the individual observations of moisture conservation gave the following results:

Variance attributable to	May-June		July-August	
	D.f.	Mean Square	D.f.	Mean Square
Linear effect of precipitation	1	26.3187**	1	19.0990**
Additional effect, length of obs. period	1	.9752**	1	1.0610**
Additional effect, antecedent pptn.	1	.2126*	1	.0544
Additional effect, time factor within periods	1	.2646*	1	.2525*
Additional quadratic effect of pptn.	1	.3562**	1	.2838**
Residual	59	.0427	64	.0358

**Exceeds mean square residual, 5% level of significance.*
***Exceeds mean square residual, 1% level of significance.*

The mean square residual is of the same order in both series of observations. Although this is small in comparison with the components accounted for by the various terms of the regression function, it nevertheless corresponds to a standard deviation of conservation of about 0.2 in. per observational period, which as noted previously had an average length of 11 days in May-June and 9 days in July-August. It is therefore desirable to ascertain whether the above mean square residuals are of the magnitude to be expected from the errors inherent in the data alone, or whether they indicate that factors exerting a sensible effect upon evaporation or conservation have still not been taken into account.

Errors in the data may arise from a variety of causes, of which the following may be mentioned specifically. (i) Differences in the change in weight recorded for replicate tanks during the same period of observation, whether due to actual variations in moisture retention, or simply to errors in weighing. (ii) Discrepancies between periods arising from the fact that the hour of weighing the tanks did not coincide with that of reading the rain-gauge. These could only occur when rain fell between the reading of the gauge and the weighing of the tanks, and in view of the fact that in most instances no precipitation whatsoever was recorded on the day on which the weighing took place, they could not have been a major source of error. It is almost certain, however, that some slight irregularities were introduced in this way. (iii) Possible discrepancies in weight between periods, arising from losses of surface soil through wind action. It might be thought that these would be included in (i) above, but this would be the case only in so far as such occurrences affected replicate soil tanks differentially.

Quantitative information is available respecting only errors of type (i). An analysis of the variance of the individual changes in tank weights for 16 periods selected at random from the total number available led to the deduction of a mean square error, based on 72 degrees of freedom, of 0.04437 per tank per period, in terms of inches of moisture. The mean square error of an average of four tanks (the minimum number observed in any period) would thus be 0.01110, i.e., about one-quarter of the mean square residuals shown on page 394 above. Even after allowing for the probable additional effect of random errors arising from sources (ii) and (iii) therefore, it seems likely that the specification of moisture conservation by the regression formulae is still susceptible of some refinement. Such a conclusion is readily reconcilable with the fact that whereas seasonal effects have been recognized in the present analysis only by the subdivision of the data into two arbitrary categories, in actuality seasonal changes, such as those in soil temperature, proceed in a continuous and gradual manner. Even with this limitation, however, Equations (V) yield multiple correlation coefficients of $R = 0.96$ and 0.95 with the observed conservation, a result which must be regarded as at least moderately satisfactory.

Seasonal Trend

The statistical significance of the seasonal factors affecting the relation between precipitation and conservation is readily demonstrable by the following analysis of variance of conservation, performed on the combined series of observations.

Variance due to	D.f.	Mean sq.	F.
Average regression function	5	6.5155	166.64***
Departure of mean conservation for May-June and July-Aug. respectively from average regression function	1	16.8239	430.28***
Differences between corresponding coefficients in separate regression functions for May-June and July-Aug.	5	.0702	1.80
Residual deviations of individual observations from separate regression functions	123	.0391	

*** *Exceeds mean sq. residual, 0.1% level of significance.*

The discrepancy is for the most part attributable to the reduced average conservation of similar amounts of rain in July-August. Apart from this, the aggregate differences between corresponding regression coefficients yield a variance ratio (3) of only 1.80. This attains approximately the 10% level of significance, and is thus perhaps suggestive of some slight degree of divergence, but does not permit its reality to be asserted with any degree of confidence.

It is likely that the departure of the conservation data for May-June and for July-August from the same regression plane is due to the effect of the seasonal trend of soil temperature on evaporation. However, even had there been no modification of the regression function, the ratio of conservation to precipitation would still have been somewhat less favourable in the latter half of the season owing to the less frequent occurrence of heavy rains which, as will be seen below, are most efficiently conserved. Table II shows a statistical classification of the individual daily rainfalls recorded during the period covered by the tank observations, from which it is to be noted that whereas 40% of the total precipitation for May-June was received in daily amounts exceeding 0.75 in., only 27% of the total for July-August fell in rains of this intensity. The preponderance of extremely light showers, common to both divisions of the season, is a disadvantageous characteristic of precipitation in the western plains region, commented upon in previous publications (4, 5), that serves to explain why, over a number of years, a "surprisingly small proportion", viz., about 30% (1) of the total precipitation was found to be actually conserved during the summer fallow period.

TABLE II

CLASSIFICATION OF DAILY AMOUNTS OF PRECIPITATION RECORDED

Period	Statistic	0.01 to 0.25 in.	0.26 to 0.50 in.	0.51 to 0.75 in.	0.76 to 1.00 in.	Over 1.00 in.
May and June	Actual frequency of occurrence	226	35	12	17	10
	Percentage of total number of days with rain	75	12	4	6	3
	Aggregate inches of rain accruing	20.32	12.20	7.69	14.99	12.48
	Percentage of total precipitation	30	18	12	22	18
July and August	Actual frequency of occurrence	233	37	22	8	7
	Percentage of total number of days with rain	76	12	7	3	2
	Aggregate inches of rain accruing	15.39	13.07	13.44	6.60	8.82
	Percentage of total precipitation	27	23	23	12	15

Inferences from Regression Function

The regression functions relating the recorded changes in soil moisture to the various observed factors enable the estimated conservation of various amounts of precipitation under any arbitrarily selected set of standard conditions to be computed. As an example, Fig. 2 shows the calculated conservation of soil moisture 10 days after the receipt of varying amounts of precipitation. It is assumed that this precipitation all occurred on the first day, and that the remainder of the period was rainless. The further assumption is made that a total of 0.50 in. of rain fell during the preceding seven days. In Equations (V), therefore, $a = 0.50$ and $d = 10$. The consistently superior retention of corresponding amounts of rainfall in the first half of the season is clearly evident from the figure. The difference in favour of May-June in this respect would have been even more pronounced had the preceding week been assumed rainless, so that the surface soil was dry at the beginning of the 10-day period. However, the value of 0.50 in. adopted for a is more nearly in accordance with the average of the quantities actually recorded. Differences in slope of the two conservation curves are slight, and as noted above, the significance of these is doubtful. In both cases the curvature is positive, indicating the retention of an increasing proportion of the larger rains, which may be assumed to penetrate to a position in the soil below the zone of most active evaporation (10).

In the absence of any precipitation at all, it is calculated that on the average 0.22 in. of moisture would be lost from the soil in May-June, and 0.31 in. in July-August. As soon as the surface soil is moistened by rainfall, however, evaporation increases, so that rain to the calculated amount of 0.36 in. in May-June and 0.46 in. in July-August would be required on the first day in order just to offset the subsequent evaporation during the 10-day period.

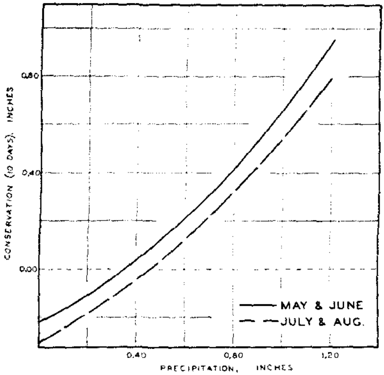

FIG. 2. *Calculated net conservation of moisture by soil 10 days after the occurrence of various one-day amounts of precipitation.*

In a sense these amounts may be regarded as the "minimum effective precipitation", although they have of course no absolute meaning apart from the specified conditions underlying their computation. Reducing the period under consideration from 10 to 5 days, for example, leads to calculated "effective minima" of 0.20 and 0.26 in. respectively, instead of the foregoing 0.36 and 0.46 in.

Fig. 3 shows the calculated percentage conservation, after 10 days, of precipitation in excess of the "effective minimum", under the conditions outlined above, and indicates the increased efficiency of the heavier rainfalls.

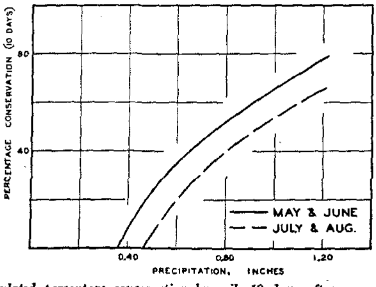

FIG. 3. *Calculated percentage conservation by soil, 10 days after occurrence, of various amounts of precipitation in excess of the one-day "effective minimum".*

This point is further illustrated in Fig. 4 which shows the computed amounts conserved at the end of 10 days (again under the same conditions as before) when 1 in. of rain is received in a single amount on the first day, in two amounts of 0.50 in. on each of the first and second days, in three amounts of 0.33 in. each on the first, second, and third days, and so on. The increased penetration and subsequent retention of the heavier rainfalls is strikingly evident. In May-June for example, 66% of a one-day rain of 1 in. is still conserved at the end of 10 days, whereas only 30% would be retained if the same amount of rain was distributed over five days. The intensity, as well as the total amount, of precipitation is thus again emphasized as a factor of prime importance in its relation to soil moisture under semi-arid conditions.

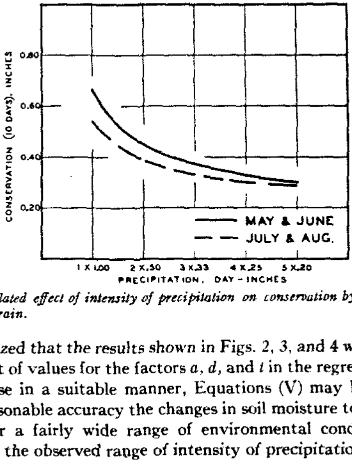

Fig. 4. Calculated effect of intensity of precipitation on conservation by soil of moisture from one inch of rain.

It will be realized that the results shown in Figs. 2, 3, and 4 were all obtained using a single set of values for the factors a, d, and t in the regression functions. By varying these in a suitable manner, Equations (V) may be employed to deduce with reasonable accuracy the changes in soil moisture to be expected in the tanks under a fairly wide range of environmental conditions. Extrapolation beyond the observed range of intensity of precipitation, namely from zero to about 1.25 in. per day, would not however be justifiable.

There must of course be some question as to the closeness with which the experimental tanks reproduce the moisture relations characteristic of an open field. Barnes (1) pointed out that initially at least the soil placed in them had been moved, stirred, and thoroughly aerated to a depth of 5 ft., and that in this process all columnar structure had been destroyed. Furthermore, the tank walls restrict the lateral movement of moisture and prohibit percolation beyond the 5-ft. depth or the upward movement of water or its vapour from below. Finally, the rims of the tanks minimize losses from surface run-off. He did not mention soil temperature, which may conceivably have been somewhat higher in the tanks, during summer at least, than at corresponding

levels in the field. It is admittedly difficult to estimate the combined effect of all these factors upon the behaviour of the tank system. Barnes himself apparently thought that on the whole conservation would be somewhat increased. The recent observations of Veihmeyer (10) on the behaviour of soil moisture in general, of Lewis (6) on the restricted role of capillary movement, and of Taggart (8) on the subject of run-off, suggest however that the resultant departure from field conditions is probably not as great as might at one time have been supposed.

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VEGETATIVE PROPAGATION OF CONIFERS

VI. HORMONE SOLUTION AND DUST TREATMENTS OF SPRUCE CUTTINGS PROPAGATED IN GREENHOUSE AND OUTSIDE FRAMES[1]

By N. H. Grace[2] and J. L. Farrar[3]

Abstract

Four monthly collections of dormant Norway spruce cuttings, January to April, were treated with indolylbutyric acid solutions and propagated in sand in a greenhouse. February and April collections gave better rooting than those of the other two months, while there was appreciably greater mortality of the March and April collections. The results with short cuttings were uniformly superior to those obtained with long cuttings. Other cuttings of the April collection were propagated outside. The short cuttings responded more favourably to outside conditions, while the long cuttings gave equally poor results inside and outside. Indolylbutyric acid treatment had no beneficial effect and was followed by reduced rooting and increased mortality at concentrations from 20 to 60 p.p.m.

Results of a late March collection of Norway, white, and black spruce cuttings treated with talc dusts containing indolylacetic acid, cane sugar, and organic mercury, indicated that Norway spruce rooted more readily than the two other species. Treatment failed to have a beneficial effect, although injury from indolylacetic acid was somewhat reduced by its combination with organic mercury.

The results of these and the other experiments reported indicate that short Norway spruce cuttings over the period from January to April root to the extent of about 50%. A May collection, an early June collection with new growth on the cuttings, and a late June collection in which the cuttings were made from new growth only, gave inappreciable rooting. Similar new-growth cuttings did, however, give some rooting when propagated in sand watered with nutrient salts.

A number of papers have reported the results of experiments on the vegetative propagation of Norway spruce (*Picea abies* Karst) cuttings (3–11, 13). Among the factors having a bearing on rooting are the stage of development at which the cuttings were taken, age of the wood in the cutting, age of the parent tree and position of the cutting on it, plant hormone chemicals and the method of application, the effects of nutrient and disinfectant treatments, and the conditions under which propagation was carried out. The present communication describes the results of a series of six experiments which provide further information on the effects of some of these factors on the rooting of spruce cuttings.

Experimental

All the spruce cuttings used in the six experiments to be described were collected from the Petawawa Forest Experiment Station, Chalk River, Ontario. The Norway spruce cuttings were obtained from a plantation of trees 19 years of age. Four monthly collections of branches, January to April, were made by the staff of the Forest Station and shipped to Ottawa by express; these

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branches provided material for Experiments 1 to 4. In Experiment 4 use was made of the March, and in Experiments 2 and 3 of the April, collections of Experiment 1.

Material for Experiments 5 and 6 was transported to Ottawa by automobile. The white spruce cuttings were taken from a somewhat younger plantation, while the black spruce were from trees of approximately the same age in a natural upland stand.

The experiments were carried on in a greenhouse during the winter months and in outdoor frames in the late spring. The propagation medium was builder's sand, and all frames were shaded with factory cotton screens. Raised benches, equipped with electric heating cables in the media, were used in the greenhouse. Outdoors the sand rested directly on the soil and no bottom heat was provided. The greenhouse temperatures were maintained around 65° F. during the winter, but, beginning in April, temperatures ranged considerably higher and averaged about 72° F.

After a suitable period in the medium the cuttings were lifted and observations made in respect of: number of cuttings dead, number callused, number rooted, number of roots, and length of roots. From the last three quantities were calculated the average number of roots per rooted cutting, the average length of root per rooted cutting, and the mean root length per group. Data on the number of cuttings dead, callused, and rooted were subjected to the inverse sine transformation to render the values suitable for analysis of variance (2).

All experiments were arranged according to the principles of experimental design with all the treatments replicated and the groups arranged in random order in the medium. All results were subjected to the analysis of variance procedure except where the data were too meagre to warrant such treatment.

Experiment 1

This experiment was designed to complement one carried out at Yale University, New Haven, Connecticut, in which four collections, October to January, of Norway spruce cuttings were treated with a series of indolylbutyric acid solutions (3, 4, 5). The present experiment involved four monthly collections, January to April, and use was made of an identical experimental arrangement, except that it was limited to cuttings with a heel. There were 10 treatments, namely, an untreated control, a water-treated control, and applications of indolylbutyric acid solutions containing 0.625, 1.25, 2.5, 5, 10, 20, 40, and 60 p.p.m. (parts of chemical to a million parts of distilled water). Cuttings were made from two length classes of twigs, the short comprising lengths from 5 to 10 cm. and the long from 10 to 20 cm. There were five cuttings per group and four replicates, making a total of 400 cuttings to the collection, with 1600 cuttings required for the experiment.

The branches were received in the third week of the month, whereupon cuttings were prepared and treated as soon as possible in the solutions for a

period of 24 hr. Forty cuttings were treated in 200 cc. of solution; after treatment they were rinsed and planted immediately.

After approximately 14 weeks the cuttings were removed and record was made of the number rooted and dead.

Experiment 2

The material and treatments were identical with those of Experiment 1, except that the treatments were increased by one concentration, namely, 100 p.p.m. indolylbutyric acid. This series of 440 cuttings was propagated outdoors.

These cuttings were given a preliminary examination approximately 14 weeks after planting, but at this time rooting was poor. Numerous living cuttings failed to root, although a number were callused. In consequence, complete data were not taken until 24 weeks after planting.

Experiment 3

Norway spruce cuttings of an April collection were treated with talc dusts containing indolylacetic acid. The experiment comprised an untreated control, treatment with talc only, and with talc containing 100 and 1000 p.p.m. of indolylacetic acid. The cuttings were short (see Experiment 1) and had a heel of 1937 wood. They were planted outdoors in the same propagation frame as the cuttings of Experiment 2. There were 15 cuttings to a group, five replicates, and the entire experiment involved the use of 300 cuttings. The cuttings were removed for counts and measurements in mid-August, approximately 15 weeks after planting.

Experiment 4

In this experiment the cuttings were treated with a factorial series of eight dusts containing indolylacetic acid at 0 and 1000 p.p.m., cane sugar at 0 and 10%, and ethyl mercuric phosphate at 0 and 50 p.p.m. The carrier dust was talc. These treatments were part of the factorial series applied to a November collection of Norway spruce and already reported (9). In the present experiment the concentration of indolylacetic acid that had been shown to have a beneficial effect previously was used. The experiment was designed to demonstrate any change in the response to chemical treatments or media. A March collection of Norway spruce was used and cuttings ranged from 5 to 9 cm. in length. These cuttings were made with a heel of old wood, whereas plain cuttings were used in the earlier experiment (9). There were ten cuttings to a group and six replicates, three in sand and three in a mixture of sand and peat (equal proportions by volume). The propagation frames were in a greenhouse.

The same eight treatments, of the factorial series, were applied to March collections of both white and black spruce (*Picea mariana* (Mill) B.S.P. and *Picea glauca* (Moench.) Voss). Cuttings of white spruce were of approximately the same lengths as the Norway, while the average for the black spruce was about 6 cm. Cuttings had a heel of old wood and there were the same number

in each group and the same number of replicates, again three in each medium. The three blocks of each of the three spruces were randomized in each medium. This statistical arrangement gave the greatest precision for the comparison of treatments within species and a lower order of precision between species. The entire experiment involved the use of 1440 cuttings, 480 of each species. The cuttings were removed and examined approximately 14 weeks after planting (April 1).

Experiment 5

Dormant Norway spruce cuttings were collected from the lower region of the tree on May 15, 1939, and treated and planted the following day. The lengths and kind of cutting and the treatments were identical with those of Experiment 4, but there were four replicates of the eight treatments, 10 cuttings to a group, and all were planted in sand outdoors in a propagation bed similar to that used in Experiments 2 and 3. The entire experiment required 320 cuttings, which were removed for examination approximately 17 weeks after planting, when the usual records were taken.

Experiment 6

This experiment involved the use of an outside propagation frame containing 24 small adjacent compartments, each with an area of 1.5 sq. ft., filled with brown sand. These 24 compartments comprised 12 pairs, one of which, chosen at random, received a weekly application of one litre of a nutrient solution (7), the others receiving water only. This experimental arrangement provided 12 replicates of the treatment with and without the nutrient solution. Within each compartment three groups of five cuttings for each experiment were placed in random order; these comprised an untreated control group, a talc control, and a treatment with 1000 p.p.m. indolylacetic acid in talc. Two separate collections of cuttings were treated in this way. Both collections were taken from the lower region of the tree, and plain cuttings, without a heel, were used. The first was made June 9, 1939, and the cuttings ranged in length from 6 to 9 cm., each bearing a new growth shoot of approximately 1 cm. in length. The second collection was made June 21 and only material of the current year's growth was utilized; these cuttings ranged from 5 to 8 cm. in length. The cuttings were examined and records taken approximately 12 weeks after planting.

Results

The statistical treatment of the data is given in detail for Experiments 1 and 2, but in the remaining four experiments it was not included.

Experiment 1. Four monthly collections of Norway spruce cuttings treated with solutions of indolylbutyric acid, and propagated in a greenhouse

Results of the analyses of variance for the number dead and rooted after treatment are given in Table I. The effects of period of collection and indolylbutyric acid treatments are highly significant. There were significant differences in the behaviour of long and short cuttings as shown by both sets of

TABLE I

EXPERIMENT 1

ANALYSIS OF VARIANCE OF RESPONSE OF FOUR MONTHLY COLLECTIONS, JANUARY TO APRIL, OF NORWAY SPRUCE CUTTINGS TREATED WITH SOLUTIONS OF INDOLYLBUTYRIC ACID

Source of variance	Degrees of freedom	Mean square — Number of cuttings — Dead	Mean square — Number of cuttings — Rooted
Rows	3	4083.21**	3133.35**
Columns	3	1525.07*	993.64
Collections	3	7421.26***	4956.39**
Error (a)	6	274.16	284.77
Long versus short cuttings	1	13411.02*	5764.71*
Interaction			
Long versus short × collection	3	879.52	476.51
Error *(b)*	12	1874.79	849.15
Treatments	9	2776.12***	1087.95**
Interaction			
Treatments × collections	27	481.36*	226.91
Treatments × long versus short cuttings	9	448.06	278.17
Treatments × long versus short × collection	27	314.51	137.15
Error *(c)*	216	245.55	166.72

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

observations. There was a significant interaction between hormone treatments and time of collection, but only for the number of cuttings dead. The nature of these significant effects is illustrated in the succeeding tables.

In Table II are given data for the number of cuttings dead following each treatment at each monthly collection. Data are given in percentage as well as in the transformed condition, and the values are averages for both short and long cuttings. The effect of month of collection, averaged over all hormone treatments, was 20% greater death for March and April than for January and February collections. The effect of indolylbutyric acid treatments, averaged over all four collections, was one of injury; the number of dead cuttings for all treatments, including water only, was greater than that for the untreated control. The three highest indolylbutyric acid concentrations caused greater death than did water only, while the results with the other treatments did not differ significantly from those with water only. The 60 p.p.m. treatment was followed by the death of 84% of the cuttings, a value greater than that of all other treatments. When the four collections were not treated, they did not differ significantly in behaviour. The water controls were progressively poorer as they were taken later in the season. The March and April collections showed a rapidly increasing death count with increasing indolylbutyric acid dosage; in January and February,

TABLE II

EXPERIMENT 1

NUMBER OF CUTTINGS DEAD OF FOUR COLLECTIONS OF NORWAY SPRUCE ON TREATMENT WITH SOLUTIONS OF INDOLYLBUTYRIC ACID

Each value in the body of the table is a mean of 8 groups of 5 cuttings

Month of collection	Kind of data	Un-treated	Water only	Concentration of indolylbutyric acid, p.p.m.								Means by month of collection
				0.625	1.25	2.5	5	10	20	40	60	
January	Transformed	35.6	46.3	57.4	59.4	41.7	34.1	43.4	56.0	52.9	65.5	49.22
	%	37.5	52.5	67.5	67.5	47.5	35.0	47.5	65.0	60.0	77.5	55.8
February	Transformed	40.1	48.2	35.5	40.1	42.1	51.5	37.1	53.1	61.2	65.8	47.45
	%	45.0	55.0	37.5	45.0	45.0	57.5	40.0	57.5	67.5	75.0	52.5
March	Transformed	38.7	54.7	49.9	60.9	70.5	68.5	75.4	77.0	76.9	85.1	65.75
	%	42.5	60.0	55.0	70.0	80.0	82.5	85.0	87.5	87.5	95.0	74.5
April	Transformed	43.9	70.5	65.3	62.7	65.5	54.4	57.8	70.5	72.1	78.5	64.12
	%	45.0	80.0	77.5	70.0	77.5	62.5	65.0	80.0	82.5	90.0	73.0
Mean over the four collections	Transformed	39.6	54.9	52.0	55.8	54.9	52.1	53.4	64.1	65.8	73.7	
	%	42.5	61.9	59.4	63.1	62.5	59.4	59.4	72.5	74.4	84.4	

Necessary differences 5% level; treatment means 7.72; interaction treatments × collection 15.45; means by month of collection 6.41.

increasing dosage did not produce injury at the same rate, and the results were more irregular.

The mortality of the short cuttings was less than that of the long; 56% of the former and 72% of the latter were dead at the end of the experiment.

In Table III are given data for the effects of treatments on the number of cuttings rooted. These are averages for all four collections and for both long

TABLE III

EXPERIMENT 1

NUMBER OF CUTTINGS ROOTED OF FOUR COLLECTIONS OF NORWAY SPRUCE ON TREATMENT WITH SOLUTIONS OF INDOLYLBUTYRIC ACID

Each value in the table is a mean of 32 groups of 5 cuttings

Kind of data	Untreated cuttings	Water only	Concentration of indolylacetic acid, p.p.m.							
			0.625	1.25	2.5	5	10	20	40	60
Transformed	18.0	9.4	20.8	13.5	18.3	16.8	18.0	8.5	9.5	2.5
%	16.9	8.8	20.0	12.5	17.5	15.6	17.5	8.8	8.1	1.9

Necessary difference, 5% level: 6.4.

and short cuttings. Treatment in water alone gave rooting below that of the untreated controls. The results with indolylbutyric acid treatments from 0.625 p.p.m. up to 10 p.p.m. did not differ from those of the untreated control. The three highest concentrations resulted in rooting below that of the untreated cuttings, while the rooting with the 60 p.p.m. treatment was also significantly below that with water only.

Data for the effects of month of collection on rooting are given in Table IV and are averages for all treatments and both lengths of cutting. A greater percentage of rooted cuttings was obtained from the February and April collections than from those made in January or March.

TABLE IV

EXPERIMENT 1

EFFECT OF MONTH OF COLLECTION ON ROOTING OF NORWAY SPRUCE CUTTINGS

Each value in the table is a mean of 80 groups of 5 cuttings

Month of collection	Number of cuttings rooted	
	Transformed data	Per cent
January	6.9	6.0
February	23.1	22.8
March	7.2	6.3
April	16.8	16.0

Necessary difference, 5% level of significance: 6.5.

Averaged over all treatments and collections, the short cuttings rooted to the extent of 17.3%, the long 8.3%.

Experiment 2. April collection of Norway spruce cuttings treated with solutions of indolylbutyric acid and propagated outside

Data from the analyses of variance of the number of cuttings dead, rooted, and alive but not rooted are given in Table V. The analyses of variance for the number and length of roots per rooted cutting and the mean root length are not given as analysis was confined to the short cuttings only, owing to indifferent rooting of the long cuttings and numerous missing values. These root counts and lengths failed to show any significant effects. Indolylbutyric acid treatment affected the number of cuttings dead, rooted, and alive but not rooted, in a significant manner. There were differences between the behaviour of long and short cuttings in relation to both the number dead and the number rooted; further, the number of rooted cuttings demonstrated the difference in the response of the two length classes of cuttings to treatment.

In Table VI are given data for the number of cuttings dead, rooted, and the number alive but not rooted. The values are averages based on both

TABLE V

EXPERIMENT 2

ANALYSIS OF VARIANCE OF RESPONSE OF AN APRIL COLLECTION OF NORWAY SPRUCE CUTTINGS PROPAGATED OUTDOORS AFTER TREATMENT WITH INDOLYLBUTYRIC ACID SOLUTIONS

Source of variance	Degrees of freedom	Mean square		
		Number of cuttings		
		Dead	Rooted	Alive but not rooted
Replicates	3	164.6	285.8	16.0
Short vs. long cuttings	1	16265.6**	8067.9**	3225.9
Error *(a)*	3	236.9	99.3	663.6
Treatments	10	2084.2**	1165.3**	485.2*
Interaction Short vs. long × treatments	10	390.3	525.3*	217.8
Error *(b)*	60	282.8	253.4	237.7

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*

TABLE VI

EXPERIMENT 2

RESPONSES OF AN APRIL COLLECTION OF NORWAY SPRUCE CUTTINGS TREATED WITH SOLUTIONS OF INDOLYLBUTYRIC ACID AND PROPAGATED OUTDOORS

Each value in the table is a mean of 8 groups of 5 cuttings

Number of cuttings	Kind of data	Un-treated	Water only	Concentration of indolylbutyric acid, p.p.m.								
				0.625	1.25	2.50	5.0	10.0	20.0	40.0	60.0	100.0
Dead	Transformed	42.0	38.5	36.9	38.7	44.7	54.4	48.2	55.8	67.2	72.3	86.7
	%	45.0	42.5	42.5	42.5	52.5	62.5	55.0	65.0	77.5	80.0	97.5
Rooted	Transformed	27.7	34.1	30.7	37.1	30.7	26.1	29.3	18.0	6.7	6.7	3.3
	%	27.5	35.0	32.5	40.0	32.5	25.0	30.0	17.5	5.0	5.0	2.5
Alive, not rooted	Transformed	27.8	24.7	28.0	21.5	16.5	14.9	18.2	19.8	16.2	14.6	0
	%	27.5	22.5	25.0	17.5	15.0	12.5	15.0	17.5	17.5	15.0	0

Necessary differences, 5% level, transformed data only; number of cuttings dead 16.8, rooted 15.9, alive but not rooted 15.4.

long and short cuttings. The three highest concentrations of indolylbutyric acid, namely, 40, 60, and 100 p.p.m., resulted in more dead cuttings than either the untreated or water control groups. None of the indolylbutyric acid treatments from 0.625 p.p.m. to 10 p.p.m. inclusive, differed from either of the control groups in respect to the number of cuttings dead. There were

no significant differences in rooting between the control groups and any of the groups treated with 0.625 to 10 p.p.m.; however, treatments from 40 to 100 p.p.m. reduced rooting below that of the controls and were below that for all other treatments, except at 20 p.p.m. The number of cuttings alive but not rooted failed to differ significantly except after 100 p.p.m. treatment, which resulted in no such living cuttings.

In Table VII are given data for the rooting of short and long cuttings after the various treatments. The short cuttings gave significantly better rooting for both control groups and for treatment from 0.625 p.p.m. up to 10 p.p.m. indolylbutyric acid, excluding the 5 p.p.m. treatment. For treatments from 20 up to 100 p.p.m. of indolylbutyric acid, injury was marked and there was no significant difference in the response of the two lengths of cutting.

TABLE VII

EXPERIMENT 2

DIFFERENTIAL ROOTING RESPONSE OF LONG AND SHORT NORWAY SPRUCE CUTTINGS TO TREATMENT WITH SOLUTIONS OF INDOLYLBUTYRIC ACID

Each value in the table is a mean of 4 groups of 5 cuttings

Kind of cuttings	Kind of data	Un-treated	Water only	Concentration of indolylbutyric acid, p.p.m.								
				0.625	1.25	2.50	5.0	10.0	20.0	40.0	60.0	100.0
Short	Transformed	42.1	48.2	51.7	51.1	48.2	23.1	45.3	19.6	6.7	13.3	6.7
	%	45	55	55	60	55	20	50	20	5	10	5
Long	Transformed	13.3	19.9	9.8	23.1	13.3	29.2	13.3	16.5	6.7	0	0
	%	10	15	10	20	10	30	10	15	5	0	0

Necessary difference, 5% level (transformed data) 22.5.

The short cuttings were rooted to a greater extent and there were fewer dead. Considering averages over all the treatments, 11 and 78% of the long cuttings were rooted and dead, respectively; the comparable results with short cuttings were 34% rooted and 42% dead. It is of interest to point out that of the April collection propagated in the greenhouse (Experiment 1), 10% of the long cuttings rooted and 81% were dead, while 22% of the short rooted, with 64% dead. These results suggest that there is little difference in either the rooting or death of the long cuttings, whether propagation occurs inside in a greenhouse or outside. However, outside conditions appear to be somewhat favourable for the short cuttings.

Experiment 3. April collection of Norway spruce cuttings treated with indolylacetic acid in talc dusts and propagated outside

The only significant treatment effect obtained on analysis of the data for the number of cuttings rooted was between the untreated control group, which rooted 56%, and the three talc dusted groups, which rooted approximately 22%. Indolylacetic acid treatment failed to affect the number of

cuttings rooted. Essentially the same treatment differences were shown by counts of the number of cuttings callused or rooted. While approximately 87% of the untreated cuttings were either rooted or callused, the average for the three talc dusted groups was 59%. Indolylacetic acid treatment did not affect the number of cuttings rooted, callused, or dead. However, while only 7% of the untreated cuttings were dead, approximately 20% of those dusted with talc, the average of the three talc treatments, had died during the course of the experiment.

There were no significant treatment effects shown by the data for the lengths of root per rooted cutting. However, untreated cuttings had an average of 1.8 roots per rooted cutting and the average for the three talc treatments was 1.5 roots, indolylacetic acid treatment having no effect. Significant differences on the mean root length were confined to the three talc treatments. The 100 p.p.m. indolylacetic acid treatment gave a mean root length of 10.6 mm., while the talc only and the 1000 p.p.m. gave an average of 20.2 mm.

Experiment 4. Spruce cuttings treated with talc dusts containing indolylacetic acid, cane sugar, and ethyl mercuric phosphate, and propagated in a greenhouse

All white and black spruce cuttings in the mixture of peat and sand died. About 8% of the black spruce planted in sand callused and none rooted, and only 7% of the white spruce cuttings in sand were callused and one cutting of the 240 was rooted. In consequence, it was impossible to make any statistical analysis of such meagre data, and the analyses were confined to data for the Norway spruce cuttings.

There were significant differences between the two media in respect of all counts. In sand, there were 23% of rooted cuttings, 39% of rooted and callused, and 54% dead; in the mixture of sand and peat the counts were, respectively, 6, 20, and 76%. Peat sand gave uniformly poorer results than sand only.

Data for the number of cuttings rooted failed to disclose any significant treatment effects. However, there were some significant effects shown by chemicals on the number of cuttings rooted and callused. Averaged over the results for both media, the three treatments with talc containing indolylacetic acid alone and in combination with cane sugar and organic mercury were below the treatment with talc only. The average number of cuttings rooted and callused following the three preceding treatments was 20%, while talc alone gave 45% of such cuttings. The results with the other four treatments did not differ from those with talc only. The average effect of indolylacetic acid was depressing. In its absence 35% of the cuttings were rooted and callused, and in its presence 24%, these values being averages for the results in both media and for treatments with and without organic mercury and cane sugar. In Table VIII are given data for the effects of the combination of indolylacetic acid and the organic mercurial, these being averages

for the results in both media and for treatments with and without cane sugar. In the absence of organic mercury, indolylacetic acid was injurious and reduced the number of cuttings rooted and callused, but this injurious effect of indolylacetic acid disappeared in the presence of organic mercury.

TABLE VIII

EXPERIMENT 4

INTERACTION EFFECTS OF INDOLYLACETIC ACID AND ORGANIC MERCURY ON THE NUMBER OF CUTTINGS CALLUSED AND ROOTED AFTER TREATMENT WITH TALC DUSTS CONTAINING INDOLYLACETIC ACID, CANE SUGAR, AND ETHYL MERCURIC PHOSPHATE

Each value in the table is a mean of 12 groups of 10 cuttings

Concentration of indolylacetic acid, p.p.m.	Kind of data	Concentration of organic mercury, p.p.m.	
		0	50
0	Transformed	38.7	31.4
	%	41.7	28.3
1000	Transformed	22.3	31.3
	%	19.2	28.3

Necessary difference 5% level 8.75 (transformed data).

Averaged over the entire experiment, treatments failed to affect in a significant manner the number of cuttings dead. However, the interaction between indolylacetic acid and organic mercury treatment was significant. Data for the interaction effects are given in Table IX and are averages for the number of cuttings dead in both media and treatments with and without cane sugar. The number of dead cuttings was increased by indolylacetic

TABLE IX

EXPERIMENT 4

INTERACTION EFFECTS OF INDOLYLACETIC ACID AND ORGANIC MERCURY ON THE NUMBER OF CUTTINGS DEAD AFTER TREATMENT WITH TALC DUSTS CONTAINING INDOLYLACETIC ACID, CANE SUGAR, AND ETHYL MERCURIC PHOSPHATE

Each value in the table is a mean of 12 groups of 10 cuttings

Concentration of indolylacetic acid, p.p.m.	Kind of data	Concentration of organic mercury, p.p.m.	
		0	50
0	Transformed	48.0	56.8
	%	53.3	69.2
1000	Transformed	61.2	53.6
	%	72.5	64.2

Necessary difference, 5% level 9.29 (transformed data).

acid treatment in the absence, but not in the presence, of organic mercury, a condition analogous to that shown by similar data for the number of rooted and callused cuttings.

Data for the numbers and lengths of root were based on results in sand only, owing to poor rooting in peat sand and numerous missing values. The average number of roots per rooted cutting was 1.4 and the average length of roots per rooted cutting was 52.8 mm., but none of the treatments gave significantly different results.

Experiment 5. Dormant Norway spruce cuttings collected in May and propagated outdoors

There were few rooted cuttings, only 10 out of the total of 320 cuttings planted, but 83% were still alive. The number of cuttings dead failed to disclose any significant treatment effects. The striking feature of these results is the absence of appreciable rooting, especially since the cuttings were taken only three weeks after the collections of Experiments 2 and 3, collections that gave a fairly substantial degree of rooting.

Experiment 6. Effects of nutrient salts and indolylacetic acid treatments on June collections of Norway spruce cuttings propagated outdoors

The first collection, 1938 wood with 1939 growth, showed no signs of rooting, and virtually all the new growth shoots were dead. No data were taken on this collection.

Cuttings from the second collection, in which the cuttings were entirely of 1939 growth, likewise failed to root in appreciable quantities. While about 11% of both the untreated and those treated with 1000 p.p.m. indolylacetic acid rooted, 20% of those with talc only were rooted. These results are suggestive, but are not statistically significant.

Data on the number of cuttings dead indicated very highly statistically significant differences due to both nutrient and dust treatments. While 57% of the cuttings that received nutrient were dead, the value was 84% in its absence. The effect of treatments on the number of cuttings dead, averaged over the results with and without nutrient treatment, showed that, while about 78% of both the untreated and those which received 1000 p.p.m. indolylacetic acid were dead, only 55% of the talc-treated cuttings died.

Discussion

It has been shown that the rooting of spruce cuttings is affected by a number of factors (3-11, 13). There are other difficulties in interpreting and correlating the results of any series of experiments on vegetative propagation. One of the more important complicating factors is variation of the conditions under which propagation is carried out. Conditions in the greenhouse vary from month to month, and outside propagation one summer may encounter widely different conditions from those prevailing the next. Such variations must be considered in relation to the rooting response of different monthly

collections of cuttings. Comparison and correlation of the results of experiments by one, or several, investigators are complicated by these sources of variability, which, in conjunction with the natural variability of the material, render it necessary to exercise care in drawing general conclusions. However, in spite of variability of the types mentioned, certain general trends have been indicated by the results of the six experiments herein described.

Dormant Norway spruce cuttings taken in the period October to April can be rooted in appreciable numbers when propagated in a greenhouse; this finding is in agreement with earlier reports (3–11, 13). The optimum period for rooting appears to be from November to January. Unpublished work by both authors has indicated that Norway spruce cuttings do not root readily when collected during the summer months.

Application of growth stimulating chemicals by the solution method of treating cuttings has failed to demonstrate anything but injurious effects with Norway spruce. While this conclusion is in agreement with the previous findings of the authors and of Deuber and Farrar (3, 5, 10), it is not in agreement with reports from Thimann and Delisle, and Hitchcock and Zimmerman (11, 13). Application of indolylacetic acid by the dust method to April cuttings, likewise was injurious to rooting or had no effect, though beneficial effects from dust treatments of November cuttings have been reported (9). There appears to be variation in the effects of phytohormone treatment with the time of year at which the cuttings are taken.

The propagating medium is one of the more important factors affecting the rooting of cuttings. In an experiment previously reported, a beneficial effect was suggested from the addition of peat moss to the sand ordinarily used (9). In the present Experiment 4, carried out four months later, a significantly injurious effect resulted from the mixture. Chadwick, working with *Taxus* cuttings, also found a change in the effect of media as the season progressed (1).

The results of Experiment 4, and numerous others carried out at various times of the year, indicated that black and white spruce cuttings are much more difficult to root than are those of Norway spruce. However, conditions optimum for one species are not necessarily optimum for another, and there may be conditions under which both black and white spruce cuttings will root with ease.

An interesting feature of the dust method of treating cuttings is the beneficial effect of the carrier dust (7, 11, 12). In Experiment 6, reduced mortality from treatment with talc only was not influenced by the application of nutrient salts to the propagating medium. This fact and the beneficial effect of charcoal reported by Rappaport (12) suggest that the action of the carrier dust is due to its physical properties rather than its chemical constituents. In Experiment 3, talc had an injurious effect, and this is probably another instance of the differential response to treatments under different conditions.

The favourable effects of watering new growth cuttings with a solution of nutrient salts in Experiment 6 are in agreement with the findings from similar treatment of dormant cuttings (7).

Short cuttings have rooted in greater numbers and with lower mortality than the long. This superiority of the short type of cutting has been shown consistently in all experiments at Ottawa and at the Petawawa Forest Experiment Station, Chalk River. This is in marked contrast to the superior results obtained from long cuttings by Deuber and Farrar at New Haven, Connecticut (3–5).

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CHROMOSOME NUMBER AND BEHAVIOUR IN A PLANT BREEDER'S SAMPLE OF PENTAPLOID WHEAT HYBRID DERIVATIVES[1]

BY R. MERTON LOVE[2]

Abstract

Meiosis was studied in *Triticum vulgare* vars. Marquis, Marquillo, Hope, and R.L. 729, in *T. durum* var. Iumillo, and in 336 rust resistant selections from their pentaploid hybrids. R.L. 729 contains some plants that are unstable cytologically. In the fifth to seventh generation material, in fifty lines that had been subjected to rigorous selection, plants were found with 28, 38, 39, 40, 41, 42, and 43 chromosomes. Less than 42% of the plants had 42 chromosomes—the Vulgare number.

Fifty per cent of the plants were heterozygous for the arrangement of one or more chromosome segments. The chromosome aberrations detected were, in order of frequency: inversions, translocations, deficiencies, and duplications. All types of aberration were transmitted from F_7 to F_8 in some cases. One chromosome deficient for a complete arm arose from an isochromosome, and one isochromosome arose from a chromosome deficient for a complete arm.

The incidence of natural crossing in the aberrant plants is much higher than in normal wheats.

It is suggested (1) that plant breeders use as parents only those plants known to be stable cytologically; (2) that a more intensive cytological analysis of hybrid derivatives would be invaluable to the plant breeder; and (3) that the plant breeder should not overlook hybrid derivatives with specific chromosome rearrangements since such plants may be the means of breaking the linkage between "desirable" and "undesirable" genes.

Introduction

A strain of *Triticum durum* var. Iumillo ($2n = 28$) was crossed with four varieties of *Triticum vulgare* ($2n = 42$) in an attempt to transfer the full immunity to stem rust possessed by Iumillo to the Vulgare wheats. Fifty of the Vulgare-like, rust-resistant lines derived from these crosses were studied cytologically at Ottawa in 1937. This was one of several studies made on the material in that year. A summary of the cytological results (15) as well as a general outline of those of particular interest to plant breeders (22) have already been published.

Since this is probably the first cytological paper that deals with a plant breeder's sample of derivatives of pentaploid wheat hybrids, it may be of interest to record how the selections were made.

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The crosses were made by Dr. K. W. Neatby at the Dominion Rust Research Laboratory, Winnipeg, Canada, in 1930, 1931, and 1932. Since 1935, Dr. R. F. Peterson of that institution has been in charge of the project.

All hybrid generations were grown under artificially induced stem rust epidemics at the Dominion Rust Research Laboratory. According to Dr. Peterson, the "Vulgare-ness" of the hybrid plants was judged chiefly on spike and glume characters. In the early hybrid generations any plant with a high degree of rust resistance, and with spikes tending toward the Vulgare type, was saved. It was considered that the progeny of these plants would include some true Vulgare types, and that lines possessing the immunity of Iumillo, although not typical Vulgare wheats in other respects, might prove useful as breeding material. Selection for Vulgare-like spike was followed more strictly in each succeeding generation. In 1936 the 50 lines remaining in the test were virtually free from stem rust. Forty-eight of these lines were Vulgare or near-Vulgare in type and two (from Marquillo × Iumillo) were Durum-like but possessed some Vulgare characteristics and were kept because of their marked resistance to stem rust. These two lines were later found to be 28-chromosome wheats.

Material

In Table I are included details of the original crosses, the number of plants examined at first metaphase and at the young pollen tetrad stage, the number of lines in 1937, the number of F_2 plants from which these lines were derived, and the total number of F_2 plants grown.

TABLE I

MATERIAL STUDIED, AND ITS SOURCE

Cross	Generation in 1937	Number of plants studied cytologically in 1937		Number of lines in 1937	Number of F_2 plants from which the lines were derived	Total number of F_2 plants grown
		First division	Young tetrads			
Marquillo × Iumillo	F_5	102	56	13	5	4,900
Iumillo × Hope	F_6, F_7	110	63	17	3	2,400
Marquis × Iumillo	F_7	50	21	9	2	2,600
Iumillo × R.L. 729	F_6, F_7	74	35	11	4	6,000
Totals		336	175	50	14	15,900

Of the four varieties of *T. vulgare* used as parents, Marquis (R.L. 84)* is the only one that is not a derivative of a pentaploid hybrid. Hope (R.L. 209), R.L. 729, and Marquillo (R.L. 1943) are more or less rust-resistant derivatives of the crosses Marquis × Yaroslav Emmer, Pentad Durum × Marquis, and Marquis × Iumillo, respectively.

* *Rust Laboratory Accession Number.*

In 1937, 23 plants of the parent varieties were examined, this number representing from four to six plants in each variety.

In 1938, 47 plants, the progeny of 18 of the hybrid derivatives examined in 1937, were studied cytologically. Seventeen plants were aberrant and one was "normal" cytologically; the 18 plants were not selfed since the aim was to ascertain the diminution or increase, as well as the dispersion, of cytological irregularities under field conditions. The progeny of the normal plant served as a check on those of the remaining 17.

In order to obviate the necessity of repeating the cytological details, the plants used as parents for the 1938 studies are indicated in the "1937 Observations".

Methods

Twenty-five seeds of each of the 50 lines and 5 parent varieties were sown at Ottawa in 1937. The seeds were planted in rows 7 in. apart and spaced 2 in. in the rows. The seeds from the 18 plants grown in 1938 were similarly treated. Spacing of the plants results in greater tillering—an important feature when one or more tillers are taken for cytological purposes.

Anthers at the meiotic stages were fixed overnight in a mixture of absolute ethyl alcohol and glacial acetic acid (3 : 1). Wheat anthers fixed singly smear better and give much clearer division figures than those fixed in the glumes. The next day the anthers were washed in 70% ethyl alcohol and stored in vials of fresh 70% alcohol. The best grade of cork must be used to ensure against discoloration of the anthers if they are to be stored several months.

All cytological studies, except those on young pollen tetrads, were made from permanent iron-aceto-carmine smear slides. The method is a modification of the technique outlined by McClintock (18).

Staining Schedule for Smear Preparations

Only the chief departures from McClintock's smear technique will be mentioned.

(1) After the cover glass has been applied, the slide is heated over a steam bath. Steam has two advantages over a dry flame: there is a more even heat, and there is no danger of boiling the stain and thus drying out the slide.

(2) After pressure has been applied to the cover glass and the slide has been allowed to cool, both are placed in a coplin jar containing a mixture of glacial acetic acid, absolute ethyl alcohol, and xylol (3 : 1 : 1). The cover glass usually drops to the bottom of the jar in two or three minutes.

(3) Then the slide and cover glass are run through the following mixtures in coplin jars. Two or three minutes in each mixture is sufficient.

Glacial acetic acid, absolute alcohol, and xylol (1 : 1 : 1).
Absolute alcohol and xylol (1 : 1).
Absolute alcohol and xylol (1 : 1).

(4) Mount in neutral damar.

From 20 to 150 complete nuclei were analysed in each plant studied at the first meiotic divisions. For the young pollen tetrad studies, 100 pollen mother-cells were examined in a plant and the numbers of micronuclei and inversion bridges were recorded for each.

A Zeiss apo. 1.4 N.A. objective and 7 × ocular were used for photomicrographs × 560 and an apo. 1.3 N.A. for those × 1100.

1937 Observations

A. Parents

Triticum durum var. Iumillo. Each of the four plants examined at the first meiotic divisions had 28 chromosomes which regularly formed 14_{II}. No multivalents were found. In the three plants studied at the young pollen tetrad stage, the percentage of tetrads with one or two micronuclei was 0, 3 and 6. No inversion bridges were detected.

T. vulgare vars. Marquis, Marquillo, and Hope. Four, five, and six plants, respectively, were examined in the three varieties. All plants had 42 chromosomes, although other studies conducted by the author and others (5, 23) have shown aneuploid plants to be not uncommon in Marquillo and Hope. In the nine plants of the three varieties examined at the tetrad stage, the percentage of tetrads with one or two micronuclei varied from 1 to 3. No inversion bridges were found.

T. vulgare var. R.L. 729. All four plants examined had 42 chromosomes. In three plants 21_{II} was the usual configuration, but in the fourth plant most nuclei contained from two to eight univalents. Two plants, including the fourth mentioned above, were studied at the tetrad stage. In the first, 6% of the tetrads had one or two micronuclei. In the irregular plant 70% of the tetrads had from 1 to 10 micronuclei; one inversion bridge was found in eight tetrads, two bridges in two cells, and three in one cell.

B. 50 Hybrid Lines

A summary of the chromosome numbers found in the 336 plants examined in 1937 is given in Table II. Details concerning generations and number of lines are also included.

From the data included in Table II it is seen that less than 42% of the plants examined contained 42 chromosomes—the Vulgare number. In the two crosses for which material from two generations was available it is obvious that the F_7 showed a decided improvement over the F_6 in respect of the increase in the percentage of plants with 42 chromosomes. It should be remarked that the 12 plants with 28 chromosomes were confined to two lines, and that all plants examined in the two lines had 28 chromosomes. In five other lines (two lines in Iumillo × R.L. 729 F_7, and one in each of Iumillo × Hope F_7, Iumillo × R.L. 729 F_6, and Marquillo × Iumillo F_6) no aneuploids were found—all plants examined having 42 chromosomes, but of course this does not eliminate the possibility that these lines contained some plants with

TABLE II

CHROMOSOME NUMBERS FOUND IN THE 50 LINES

Material	Number of lines	Frequency of plants with the chromosome numbers							Total number of plants	Percentage of plants with 42 chromosomes
		28	38	39	40	41	42	43		
Marquillo × Iumillo F_4	13	12	2	14	8	19	47	—	102	46.0
Iumillo × Hope F_6	5	—	1	4	14	8	1	—	28	3.5
Iumillo × Hope F_7	12	—	—	9	26	25	21	1	82	25.6
Marquis × Iumillo F_7	9	—	—	—	1	22	26	1	50	52.0
Iumillo × R.L. 729 F_4	7	—	—	—	1	22	21	2	46	45.6
Iumillo × R.L. 729 F_7	4	—	—	—	—	2	24	2	28	85.7
Totals	50	12	3	27	50	98	140	6	336	41.6

more or less than the Vulgare number of chromosomes. None of the five lines was free from chromosomal aberrations.

In 12 lines no 42-chromosome plants were found. While some of these lines may have contained euploid plants that were not examined, the chromosome numbers found in others make it seem unlikely that any of the plants in them have the Vulgare number (e.g., Line 15 with one 39- and six 40-chromosome plants; Line 16 with four 40-chromosome plants; Lines 22 and 23 with one 39- and four 40-chromosome plants each; and Line 42 with one 38- and six 39-chromosome plants).

The following chromosomal aberrations were found at the first meiotic division:

(a) A chromosome deficient for one complete arm (but never without its "normal" homologue) in 14 plants,

(b) An isochromosome (having a deficiency of one arm and a duplication of the other) in 8 plants,

(c) An association of four chromosomes forming a chain in 16 plants,

(d) An association of four chromosomes forming a ring in 10 plants,

(e) Two associations of four chromosomes (at least one forming a ring) in 2 plants.

(f) An association of three chromosomes forming a chain in 18 plants.

(g) Two associations of three chromosomes forming chains in 2 plants.

(h) An inversion bridge and fragment in 3 plants.

The 73 aberrations were found in 55 of the 336 plants (16.4%). They were distributed among 32 of the 50 lines (Table III).

From the practical standpoint, it may be mentioned that 81 of the 140 plants with 42 chromosomes appeared to be quite stable cytologically. The 81 plants appeared in 26 of the 50 lines and represented all four crosses. From the 81 plants more than 6,000 seeds were forwarded to Dr. Peterson at the Dominion Rust Research Laboratory to be used in establishing new rust-resistant Vulgare lines. Of the 12 plants with 28 chromosomes, six were

TABLE III

DISTRIBUTION OF PLANTS WITH ABERRATIONS FOUND AT THE FIRST MEIOTIC DIVISION

Cross and generation	Total plants studied	Number of plants with chromosome aberrations	Total number of lines	Number of lines in which aberrations were found
Marquillo × Iumillo F_6	102	14	13	7
Iumillo × Hope F_6	28	4	5	3
Iumillo × Hope F_7	82	12	12	6
Marquis × Iumillo F_7	50	15	9	8
Iumillo × R.L. 729 F_6	46	6	7	5
Iumillo × R.L. 729 F_7	28	4	4	3
Totals	336	55	50	32

fertile, and 79 seeds of these plants (which had genes for the Vulgare condition of several characters (22)) were also forwarded to Dr. Peterson for the purpose of establishing partially Vulgare-like lines to be used as parents in further hybridization work.

The distribution of the plants studied at the young pollen tetrad stage is given in Table IV. Included in the table is the distribution of plants that were found to be heterozygous for one or more inverted regions, as evidenced by the presence of inversion bridges and fragments. It is seen from Table IV that 90 of the 175 plants (51.4%) were heterozygous for one or more inversions.

An inversion bridge was seen one or more times in 82 plants, as follows:

No. of tetrads in which a bridge appeared	1	2	3	4	5	6	7	8	9	11
No. of plants	29	13	14	4	7	4	5	3	2	1

Two inversion bridges were seen once in each of seven plants, and one bridge was seen in them as follows:

No. of tetrads in which one bridge appeared	0	5	7	9	14
No. of plants	2	2	1	1	1

Three bridges were seen in one tetrad of one plant, and in it nine tetrads contained one bridge. The 90 plants were distributed among 42 of the 49 lines represented in the tetrad studies (Table IV).

Five-celled "tetrads" were found occasionally in 22 plants.

The percentage of tetrads with one or more micronuclei varied from 0 to 100 in different plants (Table V). There is an increasing frequency, on the average, of irregular tetrads in plants with 0, 1, 2, and 3 univalents. But

TABLE IV

DISTRIBUTION OF PLANTS HETEROZYGOUS FOR ONE OR MORE INVERSIONS

Cross and generation	Total plants studied	Number of plants with inversions	Total number of lines	Number of lines in which inversions were found
Marquillo × Iumillo F_5	56	33	13	13
Iumillo × Hope F_8	15	6	5	4
Iumillo × Hope F_7	48	21	12	11
Marquis × Iumillo F_7	21	5	8	4
Iumillo × R. L. 729 F_6	20	16	7	7
Iumillo × R.L. 729 F_7	15	9	4	3
Totals	175	90	49	42

the great variability, from plant to plant, in the frequency of the "loss" of the univalent in plants of the 41-chromosome group is to be noted. Plants with 20 bivalents were as stable cytologically as those with 21 bivalents. There is a significantly lower percentage of irregular tetrads in plants of the 39-chromosome group with 19 bivalents and 1 univalent than in those with 18 bivalents and 3 univalents; this situation also obtains between plants of the 40-chromosome group with 20 bivalents and those with 19 bivalents and 2 univalents.

Details of the occurrence of aberrant pollen mother-cells (with less than the $2n$ number of chromosomes) in 16 of the 336 hybrid derivatives have already been published (14).

TABLE V

VARIATION IN THE PERCENTAGE OF TETRADS WITH MICRONUCLEI IN PLANTS WITH DIFFERENT CHROMOSOME NUMBERS

Percentage of tetrads with micronuclei	Frequency of plants with the chromosome numbers									Total plants
	28	38	39		40		41	42	43	
			1_I	3_I	0_I	2_I				
0 to 10	4	—	1	—	12	—	1	70	1	89
11 to 20	2	—	2	—	—	—	6	7	—	17
21 to 40	—	—	3	—	—	4	15	2	—	24
41 to 60	—	—	—	1	—	5	15	1**	1	23
61 to 80	—	—	—	3	—	4	6	—	—	13
81 to 100	—	1	—	3*	—	4	—	—	1	9
Total plants	6	1	6	7	12	17	43	80	3	175
Mean % for group	8.5	90.0	22.4	83.7	5.4	63.2	45.7	6.3	45.0	

* *Including one plant in which less than 5_I were not seen.*

** *Plant contained an isochromosome.*

Plants with 28 Chromosomes

First division. 12 plants. No multivalents were found, 14_{II} occurring quite regularly.

Tetrad. 6 plants. The percentage of tetrads with one or two micronuclei varied from 2 to 15, the mean being 8.5%. In each of three plants, an inversion bridge was detected in three pollen mother-cells.

Plants with 38 Chromosomes

First division. 3 plants. One had $17_{II} + 4_{I}$, one had $18_{II} + 2_{I}$, and the third had 1 $\text{chain}_{IV} + 16_{II} + 2_{I}$ in 25% of the metaphase nuclei.

Tetrad. 1 plant ($17_{II} + 4_{I}$). From one to six micronuclei were found in 90% of the tetrads.

Plants with 39 Chromosomes

First Division. 27 plants. Plants of this group fall into five categories with respect to the least number of univalents observed in some nuclei:

(a) 3 plants with no univalents—$1_{III} + 18_{II}$. An association of three chromosomes was seen in 5%, 10% and 25% of the nuclei in the three plants, respectively.

(b) 7 plants with one univalent—$19_{II} + 1_{I}$. In one plant (see Parent 1, "1938 Observations") the univalent was an isochromosome.

(c) 1 plant with two univalents—$1_{III} + 17_{II} + 2_{I}$. The association of three chromosomes was observed in 25% of the nuclei.

(d) 15 plants with three univalents. The typical metaphase arrangement was $18_{II} + 3_{I}$. But in two plants, four chromosomes were associated to form a chain in 3% and 6% of the nuclei. A third plant contained an unequal bivalent, i.e., one chromosome consisted of only one arm.

(e) 1 plant with five univalents—$17_{II} + 5_{I}$. Nuclei with seven and nine univalents were frequent. Cells with three univalents or less were not seen.

Tetrad. 13 plants. Five plants were heterozygous for at least one inverted region.

(a) 1 plant with $1_{III} + 18_{II}$. From one to four, but usually one or two, micronuclei occurred in 15% of the pollen mother-cells. An inversion bridge was seen in eight tetrads.

(b) 5 plants with $19_{II} + 1_{I}$. The percentage of tetrads with from one to three micronuclei varied from 10 to 31, the mean being 22.4%. An inversion bridge was seen eight times in one plant. Four 5-celled "tetrads" were seen in one plant.

(c) 5 plants with $18_{II} + 3_{I}$. The percentage of tetrads with from one to six micronuclei varied from 53 to 85, the mean being 73.0%. An inversion bridge was detected four times in one plant, six times in another and seven times in a third.

(d) 1 plant with 1 $\text{chain}_{IV} + 16_{II} + 3_{I}$. From one to six micronuclei were seen in 78% of the tetrads.

(e) 1 plant with $17_{II} + 5_{I}$. From 1 to 10 micronuclei were observed in 100% of the tetrads.

Plants with 40 Chromosomes

First division. 50 plants. Plants of this group fall into two classes in respect of the least number of univalents observed in some nuclei:

(a) 19 plants with no univalents. One plant had 1 $ring_{IV} + 18_{II}$ in 6% of the nuclei examined. In all plants 20_{II} was the typical configuration, although as expected two or more univalents were sometimes observed. (See Parents 2, 3, 4, and 5.)

(b) 31 plants with two univalents. In one plant, 4% of the nuclei had 1 $chain_{IV} + 17_{II} + 2_{I}$, but the usual configuration in all was $19_{II} + 2_{I}$. In two plants, one of the univalents was an isochromosome.

Tetrad. 29 plants. Sixteen plants were heterozygous for at least one inversion.

(a) 11 plants with 20_{II}. The percentage of tetrads with one or two micronuclei varied from 0 to 9, the mean being 5.9%. An inversion bridge was seen once in two plants, twice in two, and three times in a fifth plant. In a sixth plant two bridges were seen in one tetrad. One or two 5-celled "tetrads" were observed in three plants.

(b) 1 plant with 1 $ring_{IV} + 18_{II}$. No micronuclei were seen in 100 tetrads.

(c) 16 plants with $19_{II} + 2_{I}$. The percentage of tetrads with from one to six micronuclei varied from 21 to 99, the mean being 62.2%. An inversion bridge was seen one, three, four, five, and seven times in three, two, one, one, and two plants, respectively. One or two 5-celled "tetrads" were observed in three plants.

(d) 1 plant with 1 $chain_{IV} + 17_{II} + 2_{I}$. From one to six micronuclei were found in 80% of the tetrads. An inversion bridge was detected nine times.

Plants with 41 Chromosomes

First division. 98 plants. Plants of this group fall into three classes with respect to the least number of univalents observed in some nuclei:

(a) 6 plants with no univalents. In one plant an association of three chromosomes was seen in 7 of the 21 nuclei examined; an association of three and one of four chromosomes (both chains) occurred in one nucleus. In five plants an association of three chromosomes was seen in 6%, 20%, 20%, 22%, and 44% of the nuclei, respectively. In all six plants, from one to nine unpaired chromosomes were observed in some nuclei.

(b) 90 plants with one univalent (one of them Parent 7). In these plants the usual arrangement was $20_{II} + 1_{I}$. Four chromosomes formed a ring or figure-of-eight in 10% of the nuclei of one plant. A chain of four chromosomes appeared in 3%, 3% and 5% of the nuclei in three plants, respectively. In another plant 5% of the nuclei contained a chain of four chromosomes and 7% a chain of three chromosomes. In five plants there was an unequal

bivalent (see Parent 6). In three plants the univalent was an isochromosome.

(c) 2 plants with two univalents. In 3% and 4%, respectively, of the nuclei in the two plants an association of three chromosomes was seen. Nuclei with less than two unpaired chromosomes were not found.

Tetrad. 43 plants. Twenty-four plants were heterozygous for one or more inversions.

(a) 1 plant with $1_{III} + 19_{II}$. From one to three micronuclei were found in 34% of the tetrads. An inversion bridge was detected in five pollen mother-cells.

(b) 1 plant with 1 $chain_{IV} + 1_{III} + 17_{II}$. From one to five micronuclei were seen in 21% of the tetrads. An inversion bridge was seen three times.

(c) 2 plants with 1 $chain_{IV} + 18_{II} + 1_{I}$. From one to six micronuclei were observed in 31% and 54% of the tetrads, respectively. In the first plant an inversion bridge was seen five times. In the second plant one bridge was seen in five tetrads and two bridges were seen in two tetrads.

(d) 39 plants with $20_{II} + 1_{I}$. The percentage of tetrads with from one to four micronuclei ranged from 5 to 74, the mean being 42.1%. An inversion bridge was detected once in seven plants, twice in two plants, three times in two plants, six times in one plant, seven times in two plants, eight times in one plant, and nine times in one plant. Two inversion bridges were seen once in each of three plants (5, 7, and 9 tetrads, respectively, contained one bridge). In the nineteenth plant to show bridges, three were found in one tetrad (9 tetrads contained one bridge). One or two 5-celled "tetrads" were observed in five plants.

Plants with 42 Chromosomes

First division. 140 plants. Seven different types of plants were found:

(a) 120 plants with 21_{II}. In these plants no multiple associations of chromosomes were detected. The maximum number of univalents in different plants varied from 2 (Parent 18) to 12. Four plants (one of them Parent 15) were characterized by an unequal bivalent; in one of them the two heteromorphic chromosomes formed a bivalent in only half the nuclei. An isochromosome was present in two plants (one of them Parent 16); in each case both arms of the aberrant chromosome were homologous with one arm of its "normal" homologue.

(b) 8 plants with 1 $ring_{IV} + 19_{II}$. In four plants, respectively, the association of four appeared as a ring in 3%, 7%, 10% (Parent 11), and 20% of the nuclei. In one plant a ring of four chromosomes was found in 4%, a chain of four in 8%, and a a chain of three in 36%, of the nuclei. In the sixth plant a ring of four (14% of the nuclei), a chain of four (18%), and a chain of three chromosomes (18%) were observed (Parent 10). In the seventh plant a ring of four and a chain of four chromosomes occurred in 30 and 10% of the nuclei, respectively: this plant also contained an unequal bivalent. Finally, in one individual a ring of four and a chain of three chromosomes were observed in 21% and 45% of the nuclei, respectively (Parent 14).

(c) 6 plants with 1 chain$_{IV}$ + 19$_{II}$. In five plants, respectively, 10%, 10%, 10%, 13% (Parent 12), and 20% of the nuclei contained a chain of four chromosomes. In the sixth plant a chain of four and a chain of three chromosomes were seen in 19 and 3% of the nuclei, respectively (Parent 13).

(d) 2 plants with 2$_{IV}$ + 17$_{II}$. In the first plant a ring of four and a chain of four chromosomes (3% of the nuclei), a ring of four (30%), and a chain of four (28%) were observed. In the second plant (Parent 8), a ring of four and a chain of four chromosomes (9% of the nuclei), a ring of four (9%), a chain of four (27%), and a chain of three (9%) were observed.

(e) 1 plant with 1 ring$_{IV}$ + 1$_{III}$ + 17$_{II}$ + 1$_{I}$. A ring of four and a chain of three chromosomes (7% of the nuclei), a ring of four (14%) and a chain of three (14%) were seen. In this plant there was an unequal bivalent.

(f) 2 plants with 1$_{III}$ + 19$_{II}$ + 1$_{I}$. The association of three chromosomes was seen in 8% and 60% of the nuclei in the two plants. Associations of four chromosomes were not detected.

(g) 1 plant with 2$_{III}$ + 18$_{II}$ (Parent 9). Two associations of three were seen once, and one association of three chromosomes occurred 14 times in the 50 nuclei examined. Associations of four or more chromosomes were not observed.

Tetrad. 80 plants. Thirty-eight plants were heterozygous for one inversion and two plants were heterozygous for two inversions.

(a) 71 plants with 21$_{II}$. The percentage of tetrads with from one to seven micronuclei ranged from 0 to 29, the mean being 5.9%. An inversion bridge was detected once in 15 plants, twice in 6 plants, three times in 3 plants, four times in 2 plants, five times in 4 plants, and six times in 2 plants. Two bridges were seen once in 2 plants, in one of which one bridge was observed in 14 tetrads. One or two 5-celled "tetrads" were seen in 9 plants.

(b) 1 plant with an isochromosome. From one to four micronuclei occurred in 45% of the tetrads.

(c) 5 plants with 1 ring$_{IV}$ + 19$_{II}$. The percentage of tetrads with one or two micronuclei varied from 1 to 4, the mean being 2.5%. An inversion bridge was seen once in one plant, twice in one plant, and three times in two plants.

(d) 3 plants with 1 chain$_{IV}$ + 19$_{II}$. The percentage of tetrads with from one to seven micronuclei in the three plants was 6, 8, and 10 respectively. An inversion bridge was seen once in one plant and twice in a second plant.

Plants with 43 Chromosomes

First division. 6 plants. In one plant no multiple associations were found; from one to seven unpaired chromosomes appeared in different nuclei. The remaining plants were of three types.

(a) 3 plants with 1$_{III}$ + 20$_{II}$. A chain of three chromosomes was seen in 8%, 20%, and 33% of the nuclei in the three plants, respectively. An unequal bivalent was present in one plant.

(b) 1 plant with 1 chain$_{IV}$ + 19_{II} + 1 univalent isochromosome (Parent 17). A chain of four chromosomes was observed in 39% of the nuclei, and a chain of three (not involving the isochromosome) in 4% of the nuclei.

(c) 1 plant with 2_{III} + 18_{II} + 1_{I}. One and two chains of three chromosomes were seen in 50 and 10% of the nuclei, respectively. Associations of four or more chromosomes were not observed in the 70 nuclei examined.

Tetrad. 3 plants. Two plants were heterozygous for an inversion.

(a) 2 plants with 1_{III} + 20_{II}. Three per cent and 47% of the tetrads, respectively, contained from one to three micronuclei. In the latter plant an inversion bridge was seen in two tetrads, and one 5-celled "tetrad" was found.

(b) 1 plant with 2_{III} + 18_{II} + 1_{I}. From one to five micronuclei were observed in 85% of the tetrads. An inversion bridge was detected in eleven tetrads.

1938 Observations

(For complete details of *Parents*, see under "1937 Observations".)

Parent 1. $2n$ = 39 (19_{II} + 1 univalent isochromosome). Micronuclei were present in 17% of the tetrads.

Progeny: Two 40-chromosome plants. One had 20_{II}, including an unequal bivalent, and 11% of the tetrads had micronuclei. The second plant had 1 chain$_{IV}$ + 18_{II} in 10% of the metaphase nuclei and 10% of the tetrads were irregular. No isochromosome was detected in the two plants.

Parents 2, 3, 4, 5. $2n$ = 40 (20_{II}). In these plants from 4 to 7% of the tetrads had micronuclei.

Progeny: Three 40-chromosome plants (20_{II}) with micronuclei in from 4 to 5% of the tetrads. One 41-chromosome plant (20_{II} + 1_{I}) heterozygous for at least one inversion.

Parent 6. $2n$ = 41 (20_{II} + 1_{I}, including an unequal bivalent).

Progeny: Five 41-chromosome plants (20_{II} + 1_{I}). One plant with a univalent isochromosome had micronuclei in 68% of the tetrads. One plant was heterozygous for an inversion.

Parent 7. $2n$ = 41 (20_{II} + 1_{I}). This plant was heterozygous for an inverted region.

Progeny: Three plants, two of which had 41 chromosomes (20_{II} + 1_{I})—first division material was not available for the third plant. Two plants were heterozygous for an inversion. The percentage of tetrads with micronuclei in the three plants was 60, 42, and 19, respectively.

Parent 8. $2n$ = 42 (2_{IV} + 17_{II}). At least one association of four chromosomes was a ring.

Progeny: One 42-chromosome plant. Three and four chromosomes were associated to form chains in 6 and 12%, respectively, of the nuclei at first metaphase; eight univalents were seen once; and 54% of the tetrads were irregular. One 43-chromosome plant (1_{III} + 20_{II}). The chain of three

chromosomes appeared in 20% of the first metaphase nuclei; and 63% of the tetrads were irregular.

Parent 9. $2n = 42\ (2_{III} + 18_{II})$.

Progeny: One (probably two—first division material was lacking for the second plant) 42-chromosome plant, with three and four chromosomes associated to form chains in 10 and 30%, respectively, of the nuclei; 8% of the tetrads had micronuclei; in the second plant 4% of the tetrads were irregular. One 43-chromosome plant with a chain of three and a chain of four chromosomes (20% of the nuclei), a chain of four (20%) and a chain of three (10%); 68% of the tetrads were irregular.

Parent 10. $2n = 42$ (1 $\text{ring}_{IV} + 18_{II}$). A ring of four (14% of the nuclei), a chain of four (18%), and a chain of three (18%) were seen. A micronucleus was found in 2% of the tetrads.

Progeny: Three 42-chromosome plants, one with a chain of four in 30% of the nuclei, and two with 21 bivalents. In the two latter plants 3% and 4% of the tetrads had micronuclei.

Parent 11. $2n = 42$ (1 $\text{ring}_{IV} + 19_{II}$). One or two micronuclei appeared in 3% of the tetrads, and an inversion bridge was seen once.

Progeny: Four 42-chromosome plants (21_{II}), two of which were heterozygous for an inversion; the percentage of irregular tetrads in three of the plants was 2, 5, and 15, respectively. One 43-chromosome plant, with a chain of three and 20 bivalents in 63% of the nuclei; an inversion bridge was seen four times, and 35% of the tetrads contained micronuclei. In the sixth plant ($2n$ = ?) there was an inversion, and 19% of the tetrads were irregular.

Parent 12. $2n = 42$ (1 $\text{chain}_{IV} + 19_{II}$). A micronucleus appeared in 6% of the tetrads.

Progeny: Three 42-chromosome plants. The first had a chain of four chromosomes in 60% of the nuclei, and 4% of the tetrads were irregular. Two plants had 21 bivalents, and 2% and 6% of the tetrads had micronuclei; an inversion bridge was seen in the latter plant. The fourth plant probably had 42 chromosomes since only 6% of the tetrads were irregular.

Parent 13. $2n = 42$ (1 $\text{chain}_{IV} + 19_{II}$).

Progeny: One 41-chromosome plant with a chain of three and 19 bivalents in 19 of 20 nuclei examined; 3% of the tetrads had one micronucleus. One 42-chromosome plant in which a ring of four (15% of the nuclei), a chain of four (45%) and a chain of three chromosomes (10%) were seen; 3% of the tetrads had a micronucleus.

Parent 14. $2n = 42$ (1 $\text{chain}_{IV} + 19_{II}$).

Progeny: One 42-chromosome plant with a ring of four and a chain of three chromosomes appearing in 20 and 60%, respectively, of the nuclei; 11% of the tetrads had one or two micronuclei and an inversion bridge was seen once.

Parent 15. $2n = 42$ (21_{II}, including an unequal bivalent).

Progeny: Two 42-chromosome plants (21_{II}). One had an unequal bivalent. The other had a micronucleus in 4% of the tetrads.

Parent 16. $2n = 42$ (21_{II}). This plant had an isochromosome which occasionally paired with one arm of its normal homologue.

Progeny: One 41-chromosome plant with 20 bivalents and a univalent isochromosome. Two 42-chromosome plants, one with 21 bivalents and the other with a chain of four chromosomes in 85% of the nuclei; the percentage of irregular tetrads in the two plants was 5 and 2, respectively.

Parent 17. $2n = 43$ (1 $\text{chain}_{IV} + 19_{II}$ + 1 univalent isochromosome). A chain of four and a chain of three chromosomes (not involving the isochromosome) appeared in 39 and 4%, respectively, of the nuclei.

Progeny: Three 42-chromosome plants (21_{II}). In one, five inversion bridges and three fragments were observed in a second anaphase pollen mother-cell, which was too early to be included in the tetrad count; and 5% of the tetrads had from one to three micronuclei. The second plant had an isochromosome, and 16% of the tetrads were irregular. In the third plant, 7% of the tetrads had one or two micronuclei.

Parent 18 (Check). $2n = 42$ (21_{II}). One or two micronuclei were found in 3% of the tetrads.

Progeny: Four 42-chromosome plants (21_{II}). An average of 3.10% of the tetrads in the four plants contained one or two micronuclei.

General Considerations

Aneuploid Plants

Approximately one-half (54.8%) of the plants examined in 1937 were aneuploids, and they were found in 43 of the 50 lines. This is an indication of the problem that confronts the plant breeder in obtaining euploid plants of the desired type by phenotypic selection from heteroploid hybrids in wheats at least. The author knows of no other cytological study on comparable material, so that it is not known whether this ratio of aneuploids to euploids in *selected* descendants of pentaploid hybrids is typical.

Kihara (10) reported that the chromosome formula of most plants with more than 14 bivalents is $(14 + x)$ bivalents $+ (7 - x)$ univalents. Thus, plants with 38, 39, 40, and 41 chromosomes should show $17_{II} + 4_I$, $18_{II} + 3_I$, $19_{II} + 2_I$, and $20_{II} + 1_I$, respectively, in the first metaphase of the reduction divisions. A few that did not conform to this classification, for instance, plants with $20_{II} + 0_I$, he called "sterile combinations" because they set no seeds or the seeds germinated poorly or not at all.

Thompson and Cameron (27) found that observation of the number of univalents (in Emmer × Vulgare F_1 backcrosses to the Vulgare wheats Chinese and Marquis) was all that was necessary, since the total number of bivalents observed in each plant proved to be the same as that determined by subtracting the number of univalents from 21, e.g., if four univalents were observed the number of bivalents observed was 17 (= 21 − 4).

On the other hand, Thompson and Hollingshead (28), in a study of derivatives of *T. vulgare* × *T. dicoccum*, reported the occurrence of a 37-chromosome plant with $17_{II} + 3_{I}$, rather than the "expected" $16_{II} + 5_{I}$.

Sears (26) studied offspring of a cross of a "haploid" *T. vulgare* var. Chinese Spring × a "diploid" *T. vulgare*. He found two 41-chromosome plants with two univalents ($1_{III} + 18_{II} + 2_{I}$), and among the progeny of a 42-chromosome plant ($1_{III} + 19_{II} + 1_{I}$) he obtained a plant with 20_{II}, which "although small and lacking in vigor had normal pollen fertility and set a number of selfed seeds."

In the present study, a considerable number, 41, of the 178 plants with 38, 39, 40, and 41 chromosomes did not have the number of univalents expected under Kihara's scheme. Most of these set seeds, and several of the lines with 20_{II} have been grown on (Parents 2, 3, and 4). Of the three plants with 38 chromosomes, two had two univalents in some nuclei. Of the 27 plants with 39 chromosomes, three, seven, one, and one had 0, 1, 2, and 5 univalents, respectively. Of the 50 plants with 40 chromosomes, 19 had 0 univalents. And of the 98 plants with 41 chromosomes, six and two had 0 and 2 univalents, respectively. Thus, it is clear that no simple relation obtains between numbers of bivalents and univalents in wheats derived from pentaploid hybrids involving Iumillo. Photomicrographs of first metaphase nuclei with $19_{II} + 1_{I}$ and with 20_{II}, as well as the "expected" types with $18_{II} + 3_{I}$ and $19_{II} + 2_{I}$ have been previously published by the author (14).

The data obtained from the tetrad studies corroborate the chromosome pairing relationships found in plants with 39 and 40 chromosomes. As expected, the seven plants with $18_{II} + 3_{I}$ were much more irregular than the six with $19_{II} + 1_{I}$ (Table V). Similarly, the 17 plants with $19_{II} + 2_{I}$ were highly irregular as compared with the 12 which had 20_{II}.

The 41-chromosome plants varied greatly in the percentage of tetrads with micronuclei. In other words univalents are included in the daughter nuclei of tetrads with varying frequencies in different individuals. There can be little doubt but that the divergent speltoid and fatuoid ratios (5, 6, 7, and 19) obtained in certain 41-chromosome lines are due, in part at least, to this phenomenon.

Segmental Deficiencies

Chromosomes deficient for one complete arm were found in 14 of the 336 plants examined in 1937. Prophase configurations of aberrant chromosomes have not been seen, but the metaphase (Fig. 1) and anaphase (Fig. 2) appearance indicates strongly that the attachment is terminal. The short chromosome was transmitted from Parent 15 (21_{II}, including an unequal bivalent) to at least one of its offspring that had the same chromosome constitution as the parent.

Darlington (1) considers that "In plants generally we may say that there is no undoubted evidence of chromosomes with terminal centromeres forming

part of the permanent complement of a species". Such chromosomes have been produced by McClintock (19) and Rhoades (24) by X-raying maize plants. Love (16) has found certain speltoids of hybrid origin to be heterozygous for the loss of one complete arm of a chromosome. Further, Love (13) has shown that white chaff off-types in a golden chaff winter wheat are homozygous for such a deficiency; these plants have now bred true for five generations (unpublished data).

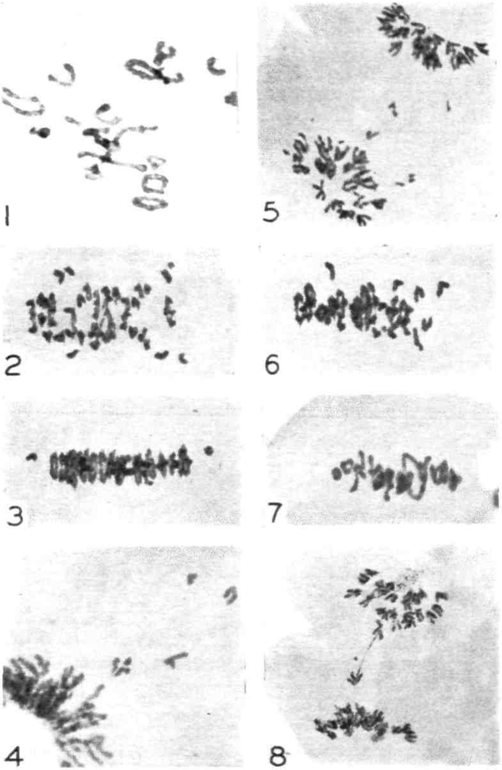
One chromosome with a terminal attachment region arose in the course of this study. Parent 1, with a univalent isochromosome, produced at least one plant (20_{II}, including an unequal bivalent) containing a chromosome with a terminal attachment region. This probably arose as a result of a break at the attachment region of the isochromosome in an embryosac mother-cell. The offspring probably resulted from a natural cross, and got the normal homologue from a nearby plant.

Segmental Duplications

Isochromosomes were found in eight plants in 1937. In four plants, these "secondary" chromosomes frequently paired with one arm of the normal homologue. In such cases disjunction at first anaphase was usually quite regular. Failure of pairing with the normal mate (Fig. 3) often resulted in breaks at the attachment region (Figs. 4 and 5) producing chromatid arms with terminal attachments, or three chromatids with a common attachment and one chromatid with no attachment. In one 43-chromosome plant, although the "extra" chromosome was an isochromosome, a "secondary trisomic" configuration was not seen.

In two instances (see Parents 16 and 17) an isochromosome was transmitted from parent to offspring. It seems that in wheat an isochromosome, and therefore deficiency-duplication gametes, can survive (see also Sears (26)). Parent 1, with an isochromosome, gave rise to a plant with a chromosome deficient for one complete arm (see above, under "Segmental Deficiencies"). A similar case has been previously reported (13).

An isochromosome appeared in one of the progeny ($2n = 41$) of Parent 6 ($2n = 41$, with an unequal bivalent). The isochromosome probably resulted from a duplication of the one arm of the deficient chromosome. The occurrence of isochromosomes in monosomic wheat has been reported by Håkansson (3), but they were then thought to be the result of crossing over in an intercalary duplication near the attachment of the "C" chromosome. Huskins and Smith (unpublished data) have obtained a chromosome consisting of the major arm of the "C" chromosome with a terminal attachment, in progeny of a plant with a "secondary" chromosome consisting of two major arms of the "C" chromosome. Rhoades (23) found that maize plants hyperploid for the short arm of chromosome V with a terminal attachment region produced a few aberrant offspring with an isochromosome. He suggested that "occasionally the 'secondary' chromosome is produced by a doubling of the half-chromosome fragment. Reduction takes place in the succeeding anaphase.



Photomicrographs of pollen mother-cells in vulgare-like derivatives of pentaploid wheat hybrids. Aceto-carmine preparations. Figs. 1 and 4 × 1100, others × 560.

FIG. 1. *Part of a first metaphase nucleus with a univalent, an association of three chromosomes, and an unequal bivalent. Note the terminal position of the attachment region in the short chromosome of the latter.* FIG. 2. *Mid-anaphase in another plant. Members of the unequal bivalent have disjoined (at right of cell).* FIGS. 3, 4, AND 5. *Behaviour of isochromosomes.* FIG. 3. *First metaphase. Isochromosome at right, normal homologue at left of cell.* FIG. 4. *Part of a first anaphase pollen mother-cell in the same plant. Three chromatid arms of the isochromosome with a common attachment, the fourth with no attachment.* FIG. 5. *Another first anaphase pollen mother-cell in the same plant. Three chromatid arms of the isochromosome with a common attachment, the fourth with a terminal attachment.* FIG. 6. *First metaphase. Note the alternate disjunction of the members of the association of four chromosomes.* FIG. 7. *First metaphase. A chain of four and a ring of four chromosomes.* FIG. 8. *First anaphase. An inversion bridge and an accompanying fragment.*

One pole receives no fragment chromosome while the other receives both fragments which have become incorporated into a single chromosome with a median attachment region."

Associations of Three and Four Chromosomes

One and two associations of three chromosomes were found in 18 and 2 plants, respectively. In six plants ($2n = 41$, with $1_{III} + 19_{II}$) the association of three was apparently due to a reciprocal translocation (the fourth member being absent in the aneuploid). The one which occurred in 44% of the nuclei may have been a trisome. One, two, three, and one plants, respectively, with 39, 41, 42, and 43 chromosomes contained one or two associations of three chromosomes, but a ring or chain of four was not found. Sears (26) found several instances of the same phenomenon.

One (Fig. 6) and two (Fig. 7) associations of four chromosomes appeared in 26 and 2 plants, respectively. The four chromosomes formed a ring in 10 and a chain in 16 plants. In some plants the association of four was frequently replaced by an association of three and a univalent, two bivalents, a bivalent and two univalents, or four univalents. It is assumed that the associations of four chromosomes are due to reciprocal translocations, the chain configurations resulting from a smaller interchange than the rings.

The reciprocal translocations may have resulted from crossing over in the F_1 hybrids between "non-homologous" chromosomes having small regions in common (compare 6, 11, 12, and 26). Multivalent configurations are frequent in F_1 pentaploid wheat hybrids (Love, unpublished data).

A chain of four chromosomes was found in parent and progeny in one case (see Parent 12), and probably in another (Parent 13) in which a chain of three was found in a 41-chromosome plant. A chain of four appeared in Parents 13 and 14 and a ring of four in one of the progeny of each. A ring (Parent 11) and a chain (Parent 17) of four chromosomes, though present in the parents, were not found in the few progeny examined. A ring of four was detected in Parents 8 and 10, but chains of four appeared in the progeny. Multiple associations were not seen in Parents 1 and 16, but chains of four appeared in one of the progeny of each; they were probably the result of natural crossing. Parent 9, with two chains of three chromosomes, gave rise to two plants, each with a chain of four chromosomes; the 42-chromosome offspring with a chain of four probably resulted from a natural cross.

The first anaphase observations of the regular disjunction of the associations of four chromosomes are corroborated by the fact that plants containing such associations have, in general, a percentage of tetrads with micronuclei no higher than have their "normal" sibs. The regular alternate disjunction of members of rings of four chromosomes in wheat has previously been reported by other workers (26, 29). In the present study only one early anaphase configuration was seen in which disjunction was definitely not alternate.

Relative Inversions

Three bridges occurred in a single tetrad in one plant, two in seven plants, and one in 82 plants. Inversion heterozygotes have been previously reported in wheat on only two occasions: in *Triticum monococcum* by Yasui (30) and in F_1 *T. dicoccum* × *T. monococcum* by Mather (17).

In three plants one inversion bridge and an accompanying fragment (Fig. 8) were seen at first anaphase. Second division material was not available for these plants so it is impossible to compare the frequencies of bridges at the two stages.

One second division bridge is the result of one (or two) cross-overs within the inverted region together with a second (or third) cross-over between the inversion and the attachment region (20). Since two second division bridges may appear in one pollen mother-cell as the result of two comparate chiasmata within the same inverted segment, the presence of two bridges in a tetrad cannot be taken as evidence for two different inversions; especially is this so since the several micronuclei usually present in a tetrad make it difficult to identify inversion fragments. The 56 plants in which one bridge was seen three times or fewer, as well as the two plants in which two bridges were detected once, may have been heterozygous for only one inverted region. But the following 32 plants were probably heterozygous for two or more inversions: the 26 plants in which one bridge was seen from four to eleven times (in 100 tetrads), the five plants in which two bridges were detected once and one bridge was seen from five to fourteen times, and the one plant in which three bridges were detected once and one bridge was seen nine times.

Inversion bridges were found in parents and progeny in two cases (see Parents 7 and 11). Unfortunately, the limitations to an exact cytological analysis of inversions in wheat are such that it is impossible to be certain that the inversions detected among the progeny of the two plants were the same ones found in the parents. No inversion bridges were detected in the 100 young pollen tetrads examined in Parent 5 ($2n = 40$) although they were in one of the progeny ($2n = 41$) which was probably the result of a natural cross. Second division material was not available for Parents 6, 8, 14, and 17; inversion bridges were detected in some of the progeny of each, and the parents may have been heterozygous for one or more inversions.

Natural Crossing

The cytological evidence, reviewed above, indicates that of the 43 progeny of the seventeen irregular plants, at least six (13.9%) resulted from natural crossing: two progeny of Parent 1, and one of the progeny of each of Parents 5, 9, 12, and 16. Goulden and Neatby (2) found that some lines of Marquillo gave as high as 50% of natural crossing, and that the frequency was associated with self-sterility. Hayes and Garber (4) reported that the incidence of natural crossing in standard varieties of wheat varied from two to three per cent. Although the number of individuals tested in the present study is small,

the results suggest that the cytological irregularities are responsible for the natural crossing that occurred.

Discussion

Disregarding irregularities in chromosome number, the 336 hybrid derivatives examined in 1937 may be divided into three categories, on the basis of chromosome behaviour:

(1) Plants comparatively stable cytologically;

(2) Plants with general irregularities, as evidenced by the failure of mating or separation of the members of any of the chromosome pairs, and even by irregularities in somatic divisions (14);

(3) Plants with irregularities confined to one or a few specific chromosomes.

While wide crosses are made in order to produce radical new combinations of genes, stable plants (Type 1 above) are necessary to establish relatively pure-breeding lines. But plants of Type 3 may be very important to the plant breeder, as well as to the worker particularly interested in general cytogenetics. It is obvious that the abnormalities (inversions, translocations, duplications, and deficiencies) are the result of combining diverse, established, viable gene combinations—and they may give rise to further, new viable combinations that are not found in the parent species or stable lines. Such plants, with specific irregularities, should not be overlooked in any breeding program since they may be the means of breaking the linkage between "desirable" and "undesirable" genes, a linkage that in the past has often proved a decided handicap in the attempt to transfer one or a few characters from one variety to another (e.g., Hope and H-44 are rust resistant wheats, but they are susceptible to "head blight" diseases (22)).

From the plant breeding standpoint, the diversity of chromosomal types derived from interspecific or intergeneric hybridization cannot be too strongly emphasized. Only rarely is the plant breeder completely successful in obtaining the desired type by selection from any one particular cross. He finds it necessary to make one or more backcrosses of the derived type on to one or the other parent, or even to cross it with a different variety. The necessity of correlating intensive cytological study with genetic analysis of such hybrids and hybrid derivatives is obvious.

Finally, in varieties such as Hope, Marquillo and R.L. 729 which are known to contain aberrant individuals, the plant breeder would be advised to use as parents plants known to be stable cytologically.

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FLUCTUATIONS IN NUMBERS OF BACTERIA IN SOIL[1]

By Norman James[2] and Marjorie L. Sutherland[3]

Abstract

Comprehensive studies on the relation of moisture, temperature, and time to the numbers of bacteria in a fallow plot were carried on over a period of 15 weeks during the summer of 1939. The data involved represent: moisture—percentages of dry weights of samples; temperature—averages of air temperature readings for three days preceding samplings; time—numbers of days from the beginning of the experiment to dates of sampling; numbers of bacteria—plate count determinations from the following:

On each of 6 dates 5 composite samples, with 48 plates from each.
On each of 6 dates 9 composite samples, with 27 plates from each.
On each of 10 dates 12 composite samples, with 27 plates from each.

On the basis of linear regression, bacteria in millions and moisture in percentage give a significant correlation for between day samples. The same is true for samples plated on the same day.

The application of multiple regression of average numbers of bacteria in the samples handled at each date on average moisture in percentage, temperature in degrees F. and time in days shows that these environmental factors account for most of the variation in numbers of bacteria in the plot during the period of this study.

The correlation between numbers of bacteria and moisture in the samples on different dates was found to be logarithmic. This relation becomes more nearly linear when the correlation is carried out on the numbers of bacteria adjusted for the factor associated with time.

Introduction

The numbers of bacteria obtained from soil by the plating method are known to fluctuate with moisture, temperature, time of sampling, and other factors, and excellent reviews have appeared in the literature. The object of this report is not primarily to add further proof of such relations, but rather to show how these gross environmental factors may mask treatment effects under consideration or introduce spurious ones; and how data may be treated to remove this masking effect in order that a better measure of the effect under consideration may be available.

Experimental

During the summer of 1939 a fallow one-hundredth acre plot on the University farm was sampled at short intervals over a period of 15 weeks in connection with three experiments on sampling and plating technique. The procedures followed were based on work reported earlier (3–5). The number of composite samples taken at random from the plot at one time ranged from 5, with 48 plates from each in the first experiment, to 9, with 27 plates from each

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in the second, and 12 with 27 plates from each in the third. The number of replications of samples, of subsamples, of dilutions from each subsample and of plates from each dilution provides the basis for a more precise estimate of the bacterial population in the plot at one time than is usual in studies of this type. At the end of the season, 22 such estimates were available, each based on the average counts from 240 plates in Experiment 1, 243 plates in Experiment 2, and 324 plates in Experiment 3, respectively. Moisture determinations were made on each sample at the time of plating. These determinations, likewise because of the number of replications at one sampling, provide fair estimates of the moisture content in the soil of the plot sampled. Unfortunately, soil temperature readings were not available for the area over the whole period concerned. Data for temperature relation studies were obtained by averaging the mean air temperature readings for the three days immediately preceding each sampling. Average air and soil temperature readings were correlated for the period extending from June 1 to August 31, and gave a correlation coefficient of .7316.

(a) Correlation Between Moisture and Numbers of Bacteria

It appeared advisable first, to examine the relation of moisture in the soil at the time of sampling to the estimate of the bacterial population from two standpoints. 1. What part of the differences among numbers of bacteria on different days can be accounted for by changes in moisture content? 2. What part of the differences among numbers from different samples from one plot on one day can be accounted for by lack of uniformity in moisture content? Analysis of covariance on the data for bacteria in millions per gram of oven-dried soil and moisture in percentage provides the following information:

Source of variance	D.f.	Variance for moisture	Variance for bacteria	Covariance	Correlation coefficient
Experiment 1. June 13 to 30. (5 samples, each of 6 days)					
*Between day samples	5	23.3809	25.3294	14.8100	0.6086
Within day samples	24	.2071	1.6475	.0413	.0707
Experiment 2. July 7 to Aug. 4. (9 samples, each of 6 days)					
Between day samples	5	344.3493	307.3084	316.6772	.9735
Within day samples	48	1.6258	2.6707	1.2328	.5906
Experiment 3. Aug. 18 to Sept. 30. (12 samples, each of 10 days)					
Between day samples	9	89.9633	113.0198	62.0062	.6155
Within day samples	110	.7789	3.9623	.5847	.3328

The Department of Soils of The University of Manitoba found the soil in the plot to have a moisture equivalent of 36.89. The data on air temperature were supplied by the same department.

* *Between day samples represent samples plated on different days. Within day samples represent those plated on the same day.*

Since the total correlation is made up of two effects, it is not included in the above analysis. The comparatively few degrees of freedom available for differences from day to day account for much greater proportions of the variance and covariance than do the larger number attributable to samples on each day.

In this study there was no reason to assume that the response of the bacterial population to changes in moisture differed in the three experiments. Accordingly, the data were combined in one analysis and the results for each experiment given appropriate weights. That is, from the three correlation coefficients for variations in moisture and numbers of bacteria from day to day, a weighted average value of z (1) was obtained, which corresponds to a correlation coefficient of 0.7806. This value approximates the accuracy of a similar correlation coefficient obtained from 16 pairs of estimates. For 14 degrees of freedom the 1% level of significance for the correlation coefficient is .6226 (6). This finding is a confirmation of previous reports that there is a significant correlation between moisture in percentage and bacteria in millions per gram of soil as estimated by the plate method.

Similarly, the correlation coefficients for moisture and bacteria on within day samples were combined; this resulted in a coefficient of .3788, with 177 degrees of freedom. In this case the 1% level of significance is .193. This shows a significant relation between moisture and numbers of bacteria in samples of soil obtained at one time from one plot—a factor that should be considered when the influence of various soil treatments or conditions on numbers of bacteria is being studied.

The difference between the correlation coefficient of .7806 for moisture and bacteria in between day samples and that of .3788 in within day samples suggests that the effect of moisture differs in the two cases. This scarcely seems reasonable. Accordingly, the data yielding the correlation coefficient of .3788 were grouped on the basis of their standard deviations. Then, new correlation coefficients were calculated for each group. The data are presented along with similar data for between day samples, as follows:

Maximum moisture range	Standard deviation	D.f.	Correlation coefficient	Level of significance
Within day samples				
1.64	0 – 0.5	24	0.0434	—
2.96	0.5 – 1.0	62	.2536	5% Pt. 0.2464
6.04	1.0 – 2.0	55	.5138	1% Pt. .3395
Between day samples				
19.34	4.7237	14	.7806	1% Pt. .6226

It is apparent that the size of the correlation coefficient is influenced by the range in moisture among the samples considered: the wider the range in moisture, the larger the coefficient. Obviously, if the samples considered were uniform in moisture the coefficient would be zero, even though there is an actual correlation between moisture and numbers of bacteria when there is variation in the moisture content among the samples used. In this case the range of moisture in the within day samples was less than 3% at 16 samplings, and not above 6.04% at the other 6, while the average moisture for between day samples varied over a range of 19.34%. This explains the apparent discrepancy between the two coefficients.

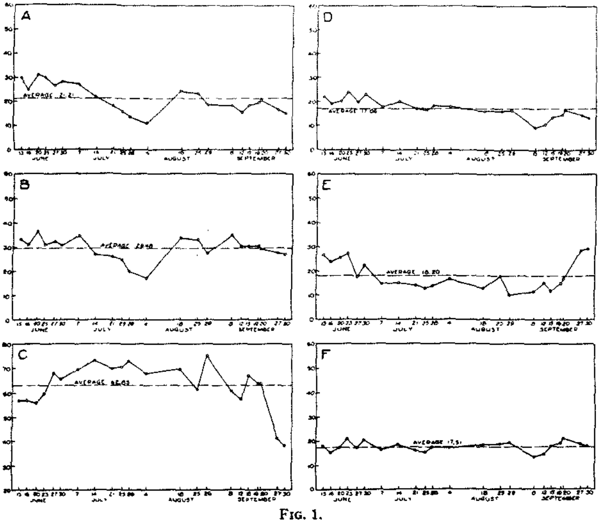

FIG. 1.

X—dates of sampling.

A... *Y—bacteria in millions per gram of oven-dried soil.*

B... *Y—moisture in percentage.*

C... *Y—average air temperature in degrees F.*

D... *Y—differences between numbers of bacteria and theoretical estimates, calculated from regression of numbers of bacteria on moisture, plotted as deviations from an estimate based on a moisture reading of 25%.*

E... *Y—differences between numbers of bacteria and theoretical estimates, calculated from regression of numbers of bacteria on moisture and temperature, plotted as deviations from an estimate based on moisture at 25% and temperature at 65° F.*

F... *Y—differences between numbers of bacteria and theoretical estimates, calculated from regression of numbers of bacteria on moisture, temperature and time, plotted as deviations from an estimate based on moisture at 25%, temperature at 65° F. and time at 55 days.*

(b) Correlation of Moisture, Temperature and Time with Numbers of Bacteria

The results of these analyses appeared consistent enough to warrant a more detailed consideration of moisture and the possible influence of additional factors on the number of bacteria in soil. Accordingly, a new set of data was prepared. Numbers of bacteria in the samples plated at one time were averaged to provide the best available estimate of the population at that time. Likewise, moisture results were averaged. These data were used without weighting for such factors as number of samples, subsamples, dilutions, and plates. Bacteria in millions per gram of oven-dried soil were plotted against dates of sampling. Similarly, moisture in percentage and air temperature in degrees F. were plotted against the same dates. The results are shown in Fig. 1–A, B, and C. The graphs for bacteria and moisture appear to be similar. Both indicate gradual decreases until Sample 12, taken August 4, followed by abrupt increases, and later downward trends. That for air temperature is quite different, showing a slight downward trend after the middle of July. On the basis of averages at each sampling, shown in Table I, the data for the season on moisture in percentage and bacteria in millions per gram of oven-dried soil give a correlation coefficient of .7564 and a regression coefficient of .9264. A theoretical estimate of the number of bacteria at each time of sampling was calculated from the moisture readings

TABLE I

Bacteria, millions	Moisture, %	Temp., °F.	Time, days	Bacteria, mult. regr.	Deviations, $A - T$	Deviations + base
29.68	33.16	56.58	1	29.09	.59	18.10
24.81	30.99	56.87	4	27.06	−2.25	15.26
30.88	36.40	55.50	8	31.11	− .23	17.28
29.65	31.00	59.47	11	26.39	3.26	20.77
26.64	32.29	68.02	15	26.88	− .24	17.27
28.16	30.71	65.30	18	25.40	2.76	20.27
26.91	34.91	69.43	25	28.08	−1.17	16.34
21.80	26.88	72.97	32	20.89	.91	18.42
17.93	26.05	69.83	39	19.67	−1.74	15.77
15.81	24.65	70.30	43	18.17	−2.36	15.15
13.73	19.96	72.93	46	14.05	− .32	17.19
10.66	17.06	67.87	53	11.21	− .55	16.96
24.31	33.95	69.63	67	23.58	.73	18.24
23.33	32.98	61.17	74	22.37	.96	18.47
18.73	27.52	75.10	78	17.28	1.45	18.96
18.34	34.93	60.60	88	22.72	−4.38	13.13
15.40	30.24	57.17	92	18.65	−3.25	14.26
18.05	30.16	67.17	95	18.09	− .04	17.47
19.58	30.59	63.60	99	18.17	1.41	18.92
20.46	29.40	63.80	100	17.11	3.35	20.86
16.70	27.69	41.12	107	15.63	1.07	18.58
15.04	27.10	38.17	110	14.96	.08	17.59
466.60	648.62	1382.62	1205			
$\bar{x}$=21.21	29.48	62.84	54.77			17.51

The base is a theoretical estimate calculated from moisture at 25%, temperature at 65° F. and time at 55 days.

on the basis of the linear regression equation. Then, in order to have moisture at about the same level as in the graph for actual numbers of bacteria (1–A), the differences between actual and theoretical estimates of the number of bacteria were plotted as deviations from a theoretical estimate based on a moisture reading of 25%. The finding is presented in Fig. 1–D. It shows that a large portion of the variation in numbers of bacteria in the plot from time to time is associated with differences in the moisture content of the samples used. Further, the line representing theoretical estimates with the effects of variation in moisture removed shows a definite downward trend.

In order to determine the variation in theoretical estimates when the effect of a second independent variable is removed along with that of moisture, multiple regression was applied to bacteria in millions, moisture in percentage and air temperature in degrees F. A multiple correlation coefficient of .7713 was obtained. This was tested for significance by calculating the F value according to Goulden (2). The result is an F value of 13.95, as compared to 5.93 at the 1% level of significance for 2 and 19 degrees of freedom. Again, theoretical estimates were calculated. The multiple regression equation with values substituted from the data follows: Y = numbers of bacteria.

$$\text{Estimated } Y = -45.5132 + .9641\ (\%\ \text{moisture}) + .6094\ (\text{degrees F.})$$

In this case, differences between actual and theoretical estimates of the number of bacteria were plotted as deviations from a theoretical estimate based on moisture at 25% and temperature at 65° F. The result is shown in Fig. 1–E and appears quite different from that in 1–A and 1–D.

In order to remove the variance causing the downward trend, apparent also in 1–E, and to examine the residual when the effect of still another variable is removed, a new multiple regression was applied involving all the data in Table I. In this case the data include a factor changing in intensity with time along with those considered previously. This factor is designated by a number for each date of sampling representing the days from the beginning of the experiment. In this analysis the multiple correlation coefficient is .9393, yielding the highly significant F value of 44.93. Here the regression equation becomes: Y = numbers of bacteria.

$$\text{Estimated } Y = 3.6706 + .8085(\%\ \text{moisture}) - .0230(\text{deg. F.}) - .0886(\text{days})$$

As before, theoretical estimates were calculated; and the differences between actual and theoretical estimates plotted, in this case, as deviations from a theoretical estimate based on moisture at 25%, temperature at 65° F. and the intensity of the factor associated with days at 55. This is presented as 1–F, and shows only minor variations in numbers of bacteria in this fallow plot when the effects of these three factors are removed by multiple regression methods.

(c) Type of Relation Between Moisture and Numbers of Bacteria

When numbers of bacteria are plotted against moisture the relation appears to be logarithmic. However, when numbers of bacteria adjusted for the factor

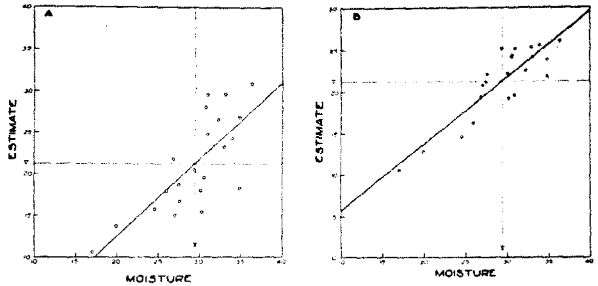

FIG. 2.

A... *X—moisture in percentage; Y—bacteria in millions.*
B... *X—moisture in percentage; Y—estimates of bacteria, representing differences between actual numbers and theoretical estimates calculated from the time factor by linear regression, plotted as deviations from a theoretical estimate based on the time factor at 55 days.*
Regression of bacteria on moisture in each case.

associated with time are plotted against the same moisture readings, the relation appears to be linear. This is shown in Fig. 2–B, where the fit of the linear regression line is much better than is the case in Fig. 2–A. In order to obtain further information on this problem, correlation coefficients were calculated from numbers of bacteria in the one case and from logarithms of the numbers in the other for the data of each of the illustrations. These are presented along with the variance for each set of numbers of bacteria. X = moisture in percentage; Y = bacteria in millions.

	2–A	2–B
Variance for Y	31.8683	17.7810
Correlation coefficient of X and Y	.7574	.8772
Correlation coefficient of X and log Y	.8029	.8898

In both cases the use of logarithms results in a slightly higher correlation coefficient. From the limited amount of data available it is not possible to draw a definite conclusion as to whether the relation is logarithmic. However, the results indicate only a slight improvement in the correlation obtained when logarithms are used on the data previously adjusted for the time factor, which also is correlated with numbers of bacteria.

Discussion

The value of the plate count as an indicator of the effects of various soil conditions or treatments depends, first, on a population sensitive to its environment, and second, on a technique capable of measuring small differences in

the population considered. A population sensitive enough to respond to differences in cropping or tillage would be expected to show the effects of marked changes in such a gross environmental factor as moisture. The finding that the refined technique used demonstrates a significant correlation between numbers of bacteria and moisture in replicate samples obtained from one plot at one time of sampling provides reason for encouragement. It indicates that the plate count technique has a place in the solution of the problems of the soil microbiologist.

While the air temperature data used in this study admittedly are of questionable value in relation to the number of bacteria in soil, the possible influence of soil temperature should be considered when the effect of another factor is under investigation. The soil temperature in a fallow plot undoubtedly would differ from that of a cropped plot in the same area. This effect might introduce an error in estimates of populations when cropped and fallow plots are involved in one experiment.

The downward trend in numbers of bacteria, which becomes more apparent when the effects of moisture and temperature are removed in the analysis, appears to indicate the presence of a factor that controls the supply of some essential food material. It should be noted that the plot under study was fallow. Consequently, there could be no effect attributable to growth of plants or the addition of organic matter. Further, the soil in the area is frozen from November to April. The mechanical effect of freezing and thawing of soil moisture may result in the disintegration of soil aggregates and the liberation of a fresh supply of the limiting nutrient in the spring.

When the environmental effects of moisture, temperature, and time are treated as constants, the data demonstrate that the number of bacteria varies within a rather narrow range. This finding, together with that concerning significant differences among estimates on samples obtained from one plot at one time when these environmental effects are not removed in the analysis, prompts a suspicion that the short-period fluctuations in bacterial populations referred to in the literature are the result of a combination of response to differences in the immediate environment from which the samples are drawn and lack of precision in the estimates.

In the light of these results, it is evident that an estimation of the effect of a treatment on the numbers of bacteria in soil includes the influence of changes in environment. These changes are not controllable under usual field conditions and may alter the result to such an extent as to obliterate differences produced by treatments, or introduce artificial ones. The influence of a treatment may be shown by a change in the effects of any or all of moisture, temperature, and time, as indicated by differences in averages, differences in the nature of regressions, or both: or it may be found in the residual after the effects of these environmental factors have been removed in the analysis.

Finally, multiple regression, accompanied by graphing of the results at various stages, may be applied to show differences not detectable by simpler analytical procedures. Additional information may be obtained from the

data, and an investigator may discover trends attributable to factors so interrelated as to be inseparable by other methods.

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THE INTERACTION OF HIGHER PLANTS AND SOIL MICRO-ORGANISMS

II. STUDY OF THE MICROBIAL POPULATION OF THE RHIZOSPHERE IN RELATION TO RESISTANCE OF PLANTS TO SOIL-BORNE DISEASES[1]

BY M. I. TIMONIN[2]

Abstract

Comparative studies of flax varieties resistant and susceptible respectively to wilt, and of tobacco varieties resistant and susceptible to black root rot, showed higher numbers of micro-organisms in the rhizosphere of the susceptible than of corresponding resistant plants. Though plants of the same variety showed considerable variation in rhizosphere population under field and greenhouse conditions, the general trend remained the same. The abundance of micro-organisms in the rhizosphere of plants of the same variety grown in plots receiving different fertilizer treatment showed relatively little difference, even though the soils varied greatly in productivity.

Numbers of micro-organisms in the rhizosphere of flax were greater when the water content of the soil was maintained at 30%, than when held at 60%, of total moisture-holding capacity. However, the microbial population in the soil distant from the roots was lower in the drier soil.

Differential counts of fungi and actinomycetes indicated that the number of colonies developing from spores or conidia comprised a small proportion of the total count. Sporulation of fungi was more profuse in soil distant from the plant than in the rhizosphere.

The contact slide method indicated a greater number of micro-organisms in the rhizosphere than in soil distant from the roots and showed differences between the rhizosphere of resistant and susceptible varieties which agreed with results from the plating method.

Introduction

Results presented in the preceding paper (26) indicate that plants in the seedling stage (3 to 16 days after planting), grown under greenhouse conditions, are capable of stimulating the microbial activity about their roots, i.e., in the rhizosphere. Furthermore it was shown that different plant varieties stimulated the growth of different groups of soil micro-organisms. In the literature are found records concerning resistance or immunity to soil-borne diseases displayed by many varieties of cultivated plants, suggesting that certain varieties through the physiological function of their roots, involving the uptake of mineral nutrients from the soil solution, and the excretion of by-products of growth or by virtue of the chemical composition of the root cells, may control the activity of the pathogens in their rhizosphere. The present investigation was planned with the object of comparing the microbial activity in the rhizosphere of root-rot resistant and susceptible varieties of cultivated plants.

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Experimental

The procedure for the rhizosphere analysis was that previously described (26) except that different media were used. For bacteria, soil extract agar, prepared according to the method of Löhnis and without added energy material, was used. For fungi the medium, prepared from soil extract, contained, in addition to 0.2 gm. of dipotassium hydrogen phosphate, 0.1 gm. of yeast extract and 1.0 gm. of glucose per litre. Immediately prior to plating, the reaction was adjusted to pH 4.8 to 5.0.

In addition to total counts of fungi, the numbers of colonies developing from spores or conidia were also estimated by the "desiccation" method of McLennan (13). Counts of actinomycetes were made on a modified Czapek's sodium-nitrate–sucrose agar (10), in which sucrose was replaced by 10 ml. of glycerol, pH = 6.7 to 6.8.

The contact slide method described by Cholodny (2) and successfully applied by Hulpoi (11) was also used, the slides being stained with phenolic rose bengal (4) for 10 min. Other special procedures will be described under separate sections.

Rhizosphere Studies of Different Crop Plants in Soils Varying in Manurial Treatment

Preliminary studies were undertaken to note the influence of cropping as compared with manurial treatment on the density of the microbial population in the rhizosphere. Rhizosphere samples from oats, clover, and mangels were taken July 11 to 17, 1938, from plots of a four-year rotation (oats, clover,

TABLE I

DENSITY OF MICRO-ORGANISMS IN RHIZOSPHERE AS INFLUENCED BY MANURIAL TREATMENT OF THE SOIL

Rhizosphere of	Yield, 1938	Fungi, thousands	Actinomycetes, millions	Bacteria, millions
Plot N				
Oats	34.4 bu.	100.8	1.9	118.1
Mangels	3.63 tons	151.8	2.4	108.2
Clover	2.58 tons	805.8	1.7	254.8
Control (soil)*		74.3	2.6	48.6
Plot X				
Oats	50 bu.	123.0	1.6	154.5
Clover	4.58 tons	819.0	1.9	286.8

Plot N = Received no fertilizer.
Plot X = 15 tons of farmyard manure applied under mangels.
** Samples obtained between the rows of mangels.*

timothy, mangels) receiving different fertilizer treatment. For 26 years Plot N had received no fertilizer, while Plot X received 15 tons of farmyard manure per acre, applied to mangels, and was in consequence much more productive than the former. With clover and oats it was virtually impossible to obtain control samples, i.e., free from roots.

The results, summarized in Table I, indicate that with the soils in question, which had become widely divergent in crop producing capacity, the type of crop was of much greater significance than the degree of fertility in determining the abundance of micro-organisms in the rhizosphere. Whereas plants of different kinds show marked variations in microbial numbers in the same soil, much lower fluctuations are noted in the rhizosphere of the same crop plant cultivated in soils that differ strikingly in fertility. The findings are in agreement with observations of Taylor and Lochhead (22) on the qualitative nature of the soil bacteria in the same plot series, which indicated that the relative incidence of the different bacterial groups was affected more by cropping than by fertilizer treatment.

The Activity of Micro-organisms as Influenced by Wilt Resistant and Susceptible Flax Varieties

A considerable amount of work has been done on the subject of resistance or immunity of plants to disease. Reviews of this literature have been published recently (14, 28). However, certain reports dealing with the resistance of cultivated plants to common root-rot diseases may be considered more closely.

Reynolds (15) found that extracts of flax plants resistant to wilt depressed the growth of *Fusarium lini* but not so the extracts from varieties of flax susceptible to this disease. He explained resistance as due to the presence of the glucoside linamarine. Dixon, Eckerson, and Link (5) explained the resistance of wheat seedlings to *Gibberella saubinetii* (Mont) Sacc. on the basis of the amount of pectins present in the cell wall. The fungus was able to infect varieties containing pectin in the cell wall, but not varieties devoid of the pectin. Rochlin (17) found that plants belonging to the Cruciferae containing glucosides such as sinigrine or gluconesturine were resistant to *Plasmodiophora brassicae*. Taubenhaus and Ezekiel (20, 21), and Ezekiel *et al.* (7, 8, 9) reported that ether extracts from root juices of monocotyledons inhibited growth of *Phymatotrichum*, whereas those from roots of susceptible dicotyledons did not.

Conant (3), studying the resistance in tobacco varieties to *Thielavia basicola*, reported that resistant varieties were able to form a cork layer in the cambial growth that inhibited the spread of mycelium in the host tissue. Susceptible varieties, however, exhibited a lag in pericyclic division behind cambial development. Ullstrup (27), however, did not find any anatomical difference in the roots of susceptible or resistant varieties of China asters.

Field Experiments

To note whether plants of the same species, but known as varieties resistant and susceptible respectively to soil-borne diseases, would exercise different influences upon the micro-organisms in their rhizospheres, samples of healthy plants of the wilt-resistant "Bison" and wilt-susceptible "Novelty" were compared. Plants had been growing in sandy loam soil, and at the time of sampling were in the flowering stage.

The results presented in Table II which are based on the average of ten plants of each variety studied, indicate that the plants of the resistant variety harboured in the rhizosphere lower numbers of soil micro-organisms than the susceptible variety.

TABLE II

NUMBERS OF MICRO-ORGANISMS IN THE RHIZOSPHERE

Varieties	Type of plant	Fungi	Bacteria	Actinomycetes
Bison	Resistant	48.5	13.1	2.1
Novelty	Susceptible	75.6	18.1	3.0

Fungi in thousands, bacteria and actinomycetes in millions per gram of moisture free soil.

Greenhouse Experiments

Further studies were made with the same varieties of flax under greenhouse conditions. The soil, obtained from the surface layer of Plot X, was passed through a ⅛-in. mesh sieve, and 200 gm. weighed out in each of 32 pots. To study the microbial population in the rhizosphere by the Rossi-Cholodny direct microscopic method, a sterile glass slide was introduced in a vertical position into the soil of each pot. Eight pots were sown with each variety of seed, four seeds to a pot, two seeds being in direct contact with the glass slide. Eight pots were kept unsown as controls. Seeds prior to planting were surface-sterilized in 1 : 1000 mercuric chloride solution for three minutes and then washed in five changes of sterile tap water.

Moisture content of the soil is considered an important factor in the development of the plant and also in the activity of micro-organisms. To study the influence of the moisture content of the soil on the activity of soil micro-organisms in the rhizosphere, the soil was adjusted in one-half of the pots to 60%, and in the second half to 30% of total moisture-holding capacity. These levels of saturation were maintained throughout the experiment by the "constant weight method" by the addition of distilled water.

The constant-capillary tension method was also used in this experiment. By this method water was continually supplied to the soil through the porous wall of the clay-cup "pressure cell". The details of the set-up and the cross section of the pressure cell are shown in Fig. 1.

The pressure cell, *a*, is a double-walled clay cup, with a porous inner wall that is in contact with the soil, and an air-proof outer wall. A rubber tube, *b*,

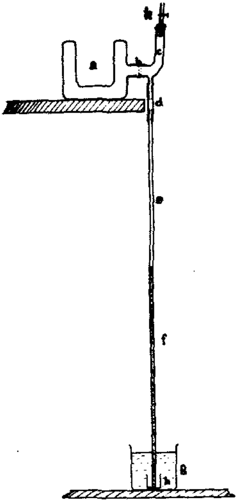

FIG. 1. *Diagram of set-up of pressure cell; explanation in the text.*

connects the pressure cell with the air trap, *c*. The air trap has a short glass tube projecting downwards, connected by means of a rubber tube, *d*, with the glass tube, *e*, 8 mm. inside diameter and 100 cm. long, and which may or may not contain a mercury column, *f*. The mercury column acts as a barostat. Glass tube *e* connects the pressure cell with the water jar, *g*. A 150 ml. glass beaker, *h*, supplies the necessary mercury to build up the mercury column and also collects it when pressure is released. When the inter-wall space of the pressure cell and the glass tube are filled with water and the rubber tube, *k*, is clamped, the capillary potential or the percentage of moisture of the soil in the pressure cell can be regulated by the height of the water and the mercury column (16).

The pressure cells were filled with air-dried soil that had been passed through a 40-mesh sieve. It was found by experiment that when the mercury column was raised in tube *e* to a height of 50 cm. the moisture content of the soil, calculated on the moisture free basis, was 14 to 15%. In the cells without the mercury column the moisture content of the soil was 27 to 28%. Two pressure cells were sown with each variety of seed, four surface sterilized seeds to the cell.

Samples for microbiological analysis were taken 15 days after planting. At the time of sampling, the glass slides were carefully removed from the soil, dried, and fixed, and the side that had been in contact with the seeds was stained with 1% phenolic rose bengal over a steam bath for 10 min.

Influence of Varieties of Flax upon the Soil Microflora

From the data presented in Table III, it is evident that the flax seedlings of both varieties supported a greater microbial population in the rhizosphere than in the soil distant from the roots. Furthermore, the data also clearly indicate the numerical difference in the microbial population in the rhizosphere of the two flax varieties. Bison, a variety resistant to wilt, supported in its rhizosphere a smaller number of micro-organisms studied, with the exception of actinomycetes, than did the susceptible variety, Novelty.

TABLE III

NUMBERS OF MICRO-ORGANISMS AS INFLUENCED BY THE MOISTURE CONTENT OF THE SOIL

Variety	Per cent of saturation	Sample from	Fungi	$\frac{R}{S}$ Ratio	Bacteria	$\frac{R}{S}$ Ratio	Actino-mycetes	$\frac{R}{S}$ Ratio
Constant weight method								
Bison (resistant)	60	R	146.2	1.39	341.5	2.49	11.9	1.3
		S	105.6		136.9		9.1	
	30	R	206.3	2.35	439.9	4.81	11.6	1.4
		S	87.8		91.4		8.1	
Novelty (susceptible)	60	R	244.1	2.29	1522.6	9.71	13.1	1.2
		S	106.8		156.7		11.0	
	30	R	500.5	6.84	2751.3	26.13	17.3	1.8
		S	73.1		105.3		9.4	
Moisture tension method								
Bison	60	R	183.2	1.34	732.4	5.21	13.9	1.5
		S	136.9		140.5		9.4	
	30	R	317.0	2.90	1103.4	10.90	13.1	1.5
		S	109.2		101.2		8.9	
Novelty	60	R	234.4	2.02	1051.9	6.90	12.6	1.4
		S	116.1		151.8		9.7	
	30	R	454.6	4.60	1439.0	12.01	13.6	1.5
		S	98.3		119.7		9.2	

R = Rhizosphere.
S = Soil distant from the roots.
$\frac{R}{S} = \frac{\text{Numbers in rhizosphere}}{\text{Numbers in the soil distant from the roots.}}$
Fungi in thousands, actinomycetes and bacteria in millions, per gram of moisture free soil.

The experiment was repeated three times; the numbers obtained varied considerably but the general trends of the density of microbial population in the rhizospheres of susceptible and resistant plants remained the same.

The marked numerical difference in the microbial accumulations in the rhizosphere of plants belonging by systematic classification to the same species may be attributed to differences in physiological function, particularly since the plants studied were free of disease and grown in disease-free soil. The results are of interest in view of the findings of Thom and Humfeld (24) who found increased numbers of micro-organisms on tobacco roots known to be infected or grown in infected soil, while fewer numbers were found on tobacco plants known to be resistant to root rot.

Influence of Moisture on the Density of Microbial Population in the Rhizosphere

The data in Table III also indicate that a lower moisture content of the soil tends to increase the density of microbial populations in the rhizosphere of susceptible as well as resistant varieties, but depresses the population in the soil distant from the roots. However, the marked differences in the density of organisms in the rhizospheres of resistant and susceptible varieties were more clearly indicated.

The constant weight method as applied for the maintenance of a certain degree of soil saturation is subject to criticism. For example, when the difference between the original weight of the pot and the weight obtained is made up by the addition of distilled water, the water tends to percolate more rapidly along the roots or by the walls of the container than in the soil itself, creating uneven distribution of moisture in the soil. To eliminate this uneven distribution of moisture, the "moisture tension method" was applied.

The experiment was started on December 12, 1938, and samples were analysed 15 days later. The greenhouse temperature during the experiment varied from 45 to 65° F., with relatively high air humidity. The results obtained with the plants grown under controlled conditions by the two methods are presented for comparison in the same table, and indicate the same general trend in the density of microbial population.

The general practice in studying microbial activity under controlled conditions is to adjust the moisture content of the soil to 50 to 60% of total moisture-holding capacity, which approximates the optimum requirement of the majority of cultivated plants. Reports by Sabinin and Minina (18), however, indicate that even in arid soil, which is practically sterile, the rhizosphere may show considerable microbial accumulations. Furthermore, Timonin (25) reported higher numbers of micro-organisms in horizons of Manitoba soils, with the moisture content below the wilting point, than in horizons of the same type of soil containing moisture available for plant growth. Results obtained by Eggleton (6) suggest that seasonal changes in moisture and temperature are not the direct causes of the seasonal changes

in numbers of bacteria in the soil, but control the growth of herbage which may be the factor responsible for the seasonal fluctuations of the soil population.

It is difficult to explain why the density of organisms in the rhizosphere is higher in the soil with 30% of total moisture-holding capacity than in that with 60%. There is no doubt, however, that the concentration of the soil solution in this experiment would be higher in the drier soil, and that this may influence the physiological function of the plant, and through it the root excretions that stimulate the growth of soil micro-organisms. Furthermore, the contact slides indicated that the formation of root hairs was more abundant in drier soil, giving a larger contact area of the roots per gram of rhizosphere soil. Consequently, a given weight of soil would contain a larger percentage of root excretion. In addition, numerous root hairs that function only for a few days would provide more easily decomposable organic matter. This, with higher concentrations of soil solution and root excretions, would presumably stimulate the activity of soil organisms, with resultant increase in numbers.

The rapidity of change in the density of the microbial population of the rhizosphere under changing moisture conditions was not studied, but the results indicate that further research in this line would probably throw some light on the seasonal fluctuations in numbers of organisms in cultivated soils.

State in Which Fungi are Recovered from Rhizosphere

The enumeration of fungi by the agar plating method provides no explanation of the precise origin of the colonies. They may have originated from spores or conidia, or from portions of mycelium. It is important, however, to know whether higher numbers in the rhizosphere are due to increased sporulation, which may be explained as a result of unfavourable conditions for growth of organisms, or to an increase in mycelial growth, which would indicate a favourable condition for fungal activity.

The data presented in Table IV indicate that colonies developing from spores or conidia represent only small fractions of the total count, namely 6 to 8%. Furthermore it is also evident that sporulation is greater in the soil distant from the roots than in the rhizosphere. The lower moisture content of the soil in the control soil tends to increase sporulation.

Several workers have presented evidence that actinomycetes are very resistant to drying. Thus Berestneff (1) was able to reisolate *A. violaceus* from inoculated ears of grain that had been stored for 10 years. Liske (12), however, stated that only conidia of saprophytic organisms were able to withstand desiccation for one and one-half years. However, the influence of desiccation *in vacuo* upon the mycelium or conidia of actinomycetes has not been studied in this investigation.

TABLE IV

NUMBERS OF ORGANISMS AS INFLUENCED BY DESICCATION

Per cent of soil saturation	Roots not desiccated		Roots desiccated		Per cent fungi colonies from spores	Per cent actinomycetes colonies from spores
	Fungi, thousands	Actinomycetes, millions	Fungi, thousands	Actinomycetes, thousands		
Bison						
60	68.8	14.1	7.7	329.1	11.0	2.3
30	185.3	16.7	10.4	381.2	5.7	2.3
Novelty						
60	166.5	10.8	13.0	445.9	7.8	4.1
30	207.5	14.6	19.3	1107.5	9.8	7.6
Soil distant from the roots						
60	45.4	6.9	5.3	552.3	11.7	8.0
30	32.1	8.4	5.7	492.2	17.8	5.9

Microbial Activity in the Rhizosphere as Recorded by *Contact Slides*

Examination of contact slides indicated noticeable differences in the abundance of the microbial accumulations in the rhizospheres and in soil distant from the roots. No distinct difference in the types of micro-organisms was observed in the rhizosphere of Bison and Novelty or in the control soil. The predominating forms of bacteria observed in all cases were short and long rods; however, coccoid forms and compact cyst-like aggregations similar to those reported by Starkey (19) were also noted.

On removal of the contact slide from the soil the rootlets in contact with the slide usually remain in the soil, leaving their imprints on the slide. These imprints are bordered with numerous root hairs most of which remained attached to the slide. It is extremely difficult to note the difference in abundance of micro-organisms about the root hairs of Bison and Novelty, (Figs. 2, 3, and 6, 7). There is, however, a noticeable difference in the density of organisms in the imprints of the rootlets of these varieties (Figs. 4, 5, and 8).

Starkey (19) noted an accumulation of bacterial colonies about fungus filaments and suggested that fungus mycelium was susceptible to bacterial attack. In this investigation, as shown in Figs. 2, 6, 9, and 12 the fungus mycelium is virtually free from bacteria. It may be that it was mere chance that fungus filaments did not come into contact with bacteria; however, the picture rather suggests the phenomenon of antagonism. It is true, however,

that the slides were kept in the soil for only 15 days, and it may be possible that after the death of mycelium the bacteria would be able to decompose it. Relatively few organisms developed on the slides that were kept in the control soil (without plants). (Figs. 10, 11, 12.)

The Activity of Micro-organisms as Influenced by Tobacco Varieties Resistant and Susceptible to Black Root Rot

To note whether the phenomenon of resistance in the case of flax varieties is also apparent with other plants, field samples of healthy tobacco plants, resistant and susceptible to black root rot, were obtained on August 2, 1938, from the same plot in adjacent rows.

Data presented in Table V indicate that the plants of the variety resistant to black root rot (R.H. 211) supported in their rhizosphere a lower number of micro-organisms than did those of the susceptible variety (C.H. 38). Data from the analysis of corn, samples of which were obtained on August 10, are

TABLE V

NUMBERS OF MICRO-ORGANISMS IN RHIZOSPHERE OF TOBACCO VARIETIES RESISTANT AND SUSCEPTIBLE TO BLACK ROOT ROT AND OF CORN

Variety	Type	Fungi	Actino-mycetes	Bacteria	Total number	Ratio of rhizosphere/soil
R.H. 211	Resistant	239.5	31.9	269.6	301.7	2.5
Control*		165.4	17.1	79.3	116.6	
C.H. 38	Susceptible	939.7	155.2	505.4	661.5	5.2
Control*		201.0	16.9	110.0	126.2	
Corn		775.1	15.4	389.2	405.8	12.4
Control*		91.3	4.5	28.2	32.8	

Fungi in thousands, actinomycetes and bacteria in millions per gram of moisture free soil.
* *Samples of control soil were obtained between the rows of plants.*

presented in the same table. Comparison of the rhizosphere-soil ratios indicate the pronounced ability of corn to stimulate microbial activity in the rhizosphere as compared with the tobacco varieties.

To verify the results obtained with plants grown under field conditions, plants of 13 tobacco varieties with different degrees of resistance to black root rot were grown under greenhouse conditions. The seeds were received from the Tobacco Division and their varietal resistance or susceptibility was unknown to the author. The seeds after surface sterilization were sown in rich greenhouse soil, and, at the fifth leaf stage, seedlings uniform in size were transplanted, one seedling per pot. The pots were filled with sandy

loam soil obtained from the surface layer of the experimental plot *N* in which tobacco had never been previously grown.

The first rhizosphere analysis of the seedlings was made 18 days after transplanting, and the second, 23 days later. The counts of micro-organisms were tabulated in order of density of microbial population in the rhizosphere. In this way three distinct groups were noted, namely, varieties with low, intermediate, and high microbial counts. When the varieties were thus classified the microbial counts were compared with the respective degree of resistance as determined by the Tobacco Division. Actually 10 out of 13 varieties were classified correctly by the microbiological method. A summary of the findings is presented in Table VI.

TABLE VI

NUMBERS OF MICRO-ORGANISMS IN RHIZOSPHERE OF TOBACCO PLANT VARIETIES WITH DIFFERENT DEGREES OF RESISTANCE TO BLACK ROOT ROT

Tobacco varieties	Fungi, thousands per gram			Actinomyces, millions per gram			Bacteria, millions per gram		
	1st Test	2nd Test	Average	1st Test	2nd Test	Average	1st Test	2nd Test	Average
Resistant—									
Havana C3X	161.6	143.9	152.8	0.8	4.4	2.6	55.7	201.2	128.5
Havana 211	57.7	*	28.9	1.0	6.4	3.7	134.7	43.6	89.2
Stand up	102.7	111.4	107.1	2.2	0.9	1.6	95.0	106.9	101.0
Stand up	112.6	41.5	77.1	2.4	7.4	4.9	96.0	174.4	135.2
Harrow Velvet	60.6	60.3	60.5	1.7	3.6	2.7	56.4	60.8	58.6
Harrow Velvet	53.4	91.6	72.5	2.0	3.3	2.7	56.3	64.0	60.2
Average	91.4	74.8	83.2	1.7	4.3	3.0	82.3	108.5	95.5
Intermediate in resistance—									
Connecticut Havana 38	97.6	96.1	96.9	1.6	4.6	3.1	83.7	93.0	88.4
Comstock Spanish	154.0	127.6	140.8	1.9	3.0	2.5	101.2	142.3	121.8
Kelley Burley	81.3	69.8	75.6	1.6	4.5	3.1	119.5	61.9	90.7
Judy Pride	73.4	87.8	80.6	2.8	4.2	3.5	117.2	106.3	111.8
Judy Price	59.2	84.8	72.0	2.2	3.1	2.7	149.5	106.0	127.8
Average	93.1	93.2	93.1	2.0	3.9	3.0	114.2	101.9	108.1
Susceptible—									
Greenwood	63.2	120.0	91.6	2.5	4.9	3.7	211.1	179.4	195.3
Greenwood	72.3	103.9	88.1	3.0	6.0	4.5	143.3	165.6	154.5
Average	67.8	112.0	90.4	2.8	5.5	4.1	177.2	172.5	174.9

* *No colonies developed on 1/1,000 dilution; numbers expressed per gram of moisture free soil.*

From the data it is quite apparent that bacteria are more sensitive to varietal differences in resistance than are fungi or actinomycetes. Fungi, especially, show considerable numerical variation. From the results obtained, however, it is felt that the adoption of certain improvements to standardize procedure, this method could be applied with advantage in aiding in the selection of varieties according to resistance to certain soil borne diseases.

Acknowledgment

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EXPLANATION OF PLATES

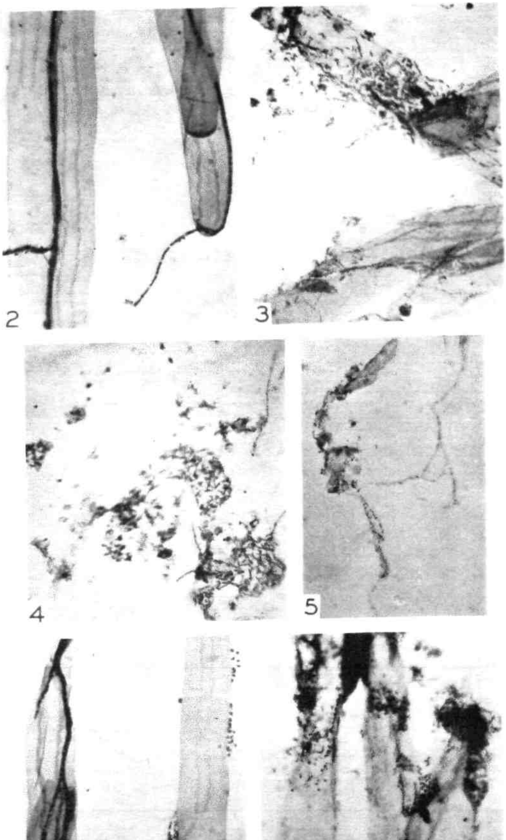
PLATE I—

FIGS. 2 – 7. *Microbial population in the rhizosphere of flax.* FIG. 2. *Root hair zone with few organisms observed. Septate branched fungus mycelium with few coccoid and short rod-shaped bacterial cells observed about fungus hyphae and root hairs. No sign of decomposition of root hairs and fungus hyphae. (Bison).* FIG. 3. *Root hair zone; same zone as in Fig. 2 but showing maximum microbial accumulation. Numerous chains of bacterial cells or actinomycete filaments and larger rod-shaped cells are predominating forms. No fungus hyphae observed. Root hairs possibly undergoing decomposition but it is also possible that they were injured by removal of slide from the soil. (Bison).* FIG. 4. *Imprint of rootlet; maximum microbial accumulation observed in this area. Actinomycete filaments and numerous rod-shaped and spherical bacterial cells. (Bison).* FIG. 5. *Same zone as in Fig. 4, showing minimum microbial accumulation. Actinomycete filaments and few deeply stained spherical and rod-shaped cells also visible. (Bison).* FIG. 6. *Root hair zone; minimum density of microorganisms observed in this area. Septate, branched, fungus hyphae surrounded by few rod-shaped bacterial cells. Colonies of rod-shaped and spherical bacterial cells but no fungus hyphae visible about the second root hair. No signs of decomposition of root hairs or fungus mycelium. (Novelty).* FIG. 7. *Root hair zone; same area as in Fig. 6 with maximum microbial accumulations. Rod-shaped bacterial cells predominant; deeply stained masses of bacterial cells. Protoplasm in the root hairs still visible. (Novelty).*

PLATE II—

FIGS 8 AND 9. *Microbial population in rhizosphere of flax.* FIG. 8. *Imprint of rootlet with maximum number of organisms. Actinomycete filaments and rod-shaped bacterial cells predominant; few spherical cells. (Novelty).* FIG. 9. *The fungus hyphae just outside the area shown in Fig. 8. No bacterial cells observed about fungus hyphae. (Novelty).*

FIGS 10 – 12. *Microbial population in the soil (control) without plants.* FIG. 10. *Maximum density of organisms observed. Colonies of long rod-shaped bacteria.* FIG. 11. *Minimum density observed on the same slide as in Fig. 10. Actinomycete filaments and few spherical cells.* FIG. 12. *Fungus hyphae also observed on the same slide, but no bacterial colonies developed about them.*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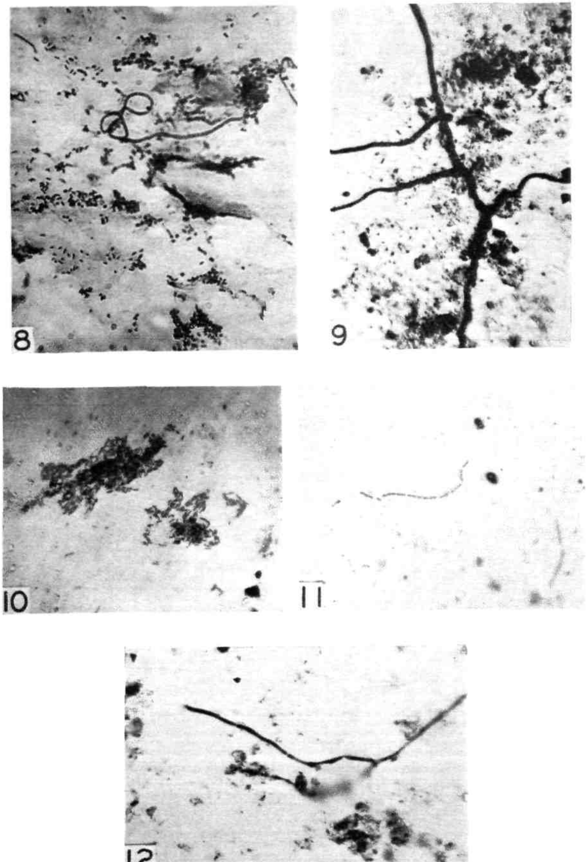
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RESPONSES OF PLANT CUTTINGS TO TREATMENT WITH NAPHTHYL ACIDS AND THEIR POTASSIUM SALTS IN A TALC CARRIER[1]

By N. H. Grace[2]

Abstract

Cuttings of one herbaceous and of four dormant woody plants were treated with a series of talc dusts containing indolylacetic acid and the acids and potassium salts of several members of the naphthyl series of root growth stimulating chemicals. The higher members of the series compared favourably with naphthylacetic acid in respect to most of the responses considered, though there were indications that naphthylacetic acid or its salt had a greater effect on the number of roots per rooted cutting. Naphthylacetic and 1-γ-naphthylbutyric members of the series were equally effective as acids or salts; however, a mixture of the isomeric 1- and 2-γ-naphthylbutyric acids was more active than the corresponding mixture of salts. Conversely, potassium naphthylhexoate appeared to have greater activity than the acid. The results suggest that pure naphthylbutyric acid, the isomeric mixture of acids, and potassium naphthylhexoate are virtually as effective as the recognized plant growth stimulating chemicals, indolyl- and naphthylacetic acids.

A noteworthy feature of the results was the beneficial effect of treatment with talc alone. The promotion of new growth and rooting of dormant cuttings were of particular interest, though most of the other criteria studied also indicated beneficial responses.

An earlier communication has shown that all the naphthyl acids from the acetic to the hexoic have a measure of physiological activity as determined by the rooting responses of solution-treated plant stem cuttings (4). The outstanding feature of the results was the activity of those members of the series with an even number of carbon atoms in the side chain. It was suggested that the higher members of the series might be particularly suited to the treatment of cuttings as they effected excellent rooting with, apparently, somewhat less danger of shock from overdosage than has been noted with the acetic member of the series. The present paper describes five further experiments in which cuttings of different plants were treated with talc dusts containing 1-naphthylacetic, 1-γ-naphthylbutyric, 1- and 2-γ-naphthylbutyric, and ε-(1-naphthyl)-hexoic acids. On this occasion, the series of dust treatments included the potassium salts of the naphthyl acids, as it was of interest to ascertain whether the relatively water soluble potassium salts were more active than the sparingly soluble long chain acids. The mixture of naphthylbutyric isomers was considered of particular interest from the practical point of view, since the mixture of the isomers is prepared more readily than the 1-γ-naphthylbutyric acid.

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Experimental

A series of 10 dusts was prepared, comprising talc and talc containing 1000 p.p.m. (parts of chemical per million parts of talc by weight) of the acids, and the potassium salts of the four naphthyl acids in amounts to provide the equivalent of 1000 p.p.m. of the acid concerned. All the dusts, including talc only, were subjected to a grinding mix operation for about 15 hr. (3). These 10 dusts, along with a control that contained no dust, provided a series of 11 treatments.

Each of the five experiments involved 352 cuttings divided into four replicated groups of eight cuttings* for each of the 11 treatments. These groups were planted in random order in the bench according to the principles of experimental design. Cuttings were sprayed with water prior to dusting to ensure uniform adhesion of a quantity of dust (15). They were then planted in brown sand in propagation frames equipped with electrical bottom heat cables. Sand temperature was maintained around 72° F., while that of the greenhouse room ranged around 65° F.

In the first four experiments, dormant woody cuttings were planted November 13 and 14, 1939. *Lonicera tartarica* L. cuttings were removed 48 days later, while those of *Sambucus canadensis* L. and *Deutzia scabra* Thunb. were removed 65 days after planting. *Hydrangea arborescens* L. cuttings were removed 78 days after planting. Cuttings of one herbaceous plant, *Iresine Lindeni* Lem., were planted February 22 and removed 30 days later. For the dormant cuttings, record was made of the number of cuttings rooted, the number showing new growth, and the number and length of roots per rooted cutting, except in the case of *Deutzia* of which, owing to a large number of roots, the length of root mass was determined instead of the root length (7). Further, where root length per rooted cutting was determined, the mean root length was calculated. In each case the weight of new growth per cutting with new growth was determined. Fresh root weights per group of eight cuttings were obtained for both *Hydrangea* and *Deutzia* cuttings. Owing to considerable secondary root development, in the case of cuttings of the latter plant, where root lengths per rooted cutting were not determined, fresh root weights per rooted cutting also were considered. Counts of the number of callused cuttings were made for *Lonicera* only, this count including all callused cuttings both with and without roots. Examination of the *Iresine* cuttings yielded data on the number of cuttings dead and rooted, the number of roots per rooted cutting, the length of root mass (7), the fresh root weight expressed in terms of root weight per rooted cutting and, finally, the fresh weight of the surviving cuttings after the roots were removed.

All data were subjected to analyses of variance. Data for all counts of numbers of cuttings were subjected to the inverse sine transformation prior to statistical analysis (1). In the experiment with *Lonicera* the rooting of the untreated and talc treated control groups was poor and the meagre data

* *The cuttings were supplied by the Federal District Commission, Ottawa, through the kindness of Mr. E. I. Wood.*

for the number and length of roots and the mean root length were not suited to analyses of variance. Statistical analysis of these three responses was confined to the remaining nine treatments in this experiment.

Results

A table of data is presented for each of the five experiments but the details of the analyses of variance are not given. The necessary differences between individual treatment means have been calculated for all responses showing some significant treatment effects.

Lonicera tartarica

Data for the responses of *Lonicera* cuttings are given in Table I. None of the untreated cuttings rooted; rooting of the talc treated controls was below the mean of all hormone treatments and there were no other significant treatment effects. All the talc-treated cuttings (with and without hormone chemical) gave a greater number of callused cuttings than the untreated control group; none of the results with chemical treatments differed significantly from those with talc alone. Indolylacetic acid treatment produced fewer roots than the average for all naphthyl treatments. Both the hexoic acid and its salt gave fewer roots than the other naphthyl compounds, which did not differ among themselves in the effects produced. The average effect of naphthyl acid or salt treatment was to produce a greater length of root per rooted cutting than that resulting from indolylacetic acid application. Treatments, neither as a whole nor on partition, attained significance in respect to the mean root length. The number of cuttings showing new growth was increased in marked manner by talc treatment. Data for the weight of new growth per cutting with new growth have been included, but they failed to reveal any significant treatment effects. The data, on the whole, fail to show any average differences between naphthyl acids or salts, and the results for the mixture of isomeric butyric acids in no instance differ from those for the pure 1-γ-naphthylbutyric acid.

Sambucus canadensis

In Table II are given data for the responses of *Sambucus* cuttings. A substantial increase in the number of rooted cuttings was noted following treatment, but this was apparently attributable to the talc rather than to the phytohormone acids or salts. Similarly, talc treatment increased the number of roots per rooted cutting. The results for both the acids and salts of the isomeric mixture of butyric acids suggest a greater number of roots than that following application of the pure chemical, but the observed differences are not statistically significant. The length of root per rooted cutting was also increased by all the talc treatments. While the groups of acids and salts failed to differ on the average, the salts of the isomeric mixture of butyric acids excelled the hexoate in respect of root length. All 10 talc treatments produced a greater mean root length than the untreated group of cuttings. Similarly, all 10 talc treatments resulted in a substantial increase in the number

TABLE I

RESPONSES OF DORMANT *Lonicera tartarica* CUTTINGS TREATED WITH TALC DUSTS CONTAINING PLANT HORMONE ACIDS AND POTASSIUM SALTS

Data are means for four groups of eight cuttings

		Controls		Treated with talc containing 1000 *p.p.m.* of									Necessary difference, 5% level
				Acids					Potassium salts of				
		Un-treated	Talc treated	Indolyl-acetic	1-naphthyl-acetic	1-γ-naphthyl-butyric	1 and 2-γ-naphthyl-butyric	ε-(1-naphthyl)-hexoic	1-naphthyl-acetic	1-γ-naphthyl-butyric	1 and 2-γ-naphthyl-butyric	ε-(1-naphthyl)-hexoic	
Number of cuttings rooted	Transformed data	0	20.7	37.7	39.5	45.0	49.3	35.3	56.3	44.3	46.8	33.9	19.0
	Per cent	0	21.9	37.5	40.6	50.0	56.3	34.4	68.8	50.0	53.1	31.3	
Number of cuttings callused	Transformed data	16.5	54.8	48.8	43.1	47.0	55.4	46.8	58.1	48.6	50.6	42.7	19.0
	Per cent	21.9	65.6	56.3	46.9	53.1	65.6	53.1	71.9	56.3	59.4	46.9	
Number of roots per rooted cutting		+	+	5.5	17.4	11.4	10.3	5.1	14.3	13.7	13.4	5.6	7.2
Length of root per rooted cutting, mm.		+	+	89	382	274	249	159	298	295	287	100	184
Mean root length, mm.		+	+	14.7	21.8	22.2	24.6	25.9	21.8	18.9	21.0	18.5	10.1
Number of cuttings with new growth	Transformed data	9.5	54.8	50.6	45.0	43.2	38.8	41.4	58.6	42.5	48.6	41.3	18.0
	Per cent	9.4	65.6	59.2	50.0	46.9	40.6	43.8	71.9	46.9	56.3	43.8	
Weight of new growth per cutting with new growth, gm.		+	0.31	0.40	0.28	0.39	0.39	0.39	0.41	0.55	0.37	0.38	

+ *Meagre data, not suited to analysis of variance.*

TABLE II

RESPONSES OF DORMANT *Sambucus canadensis* CUTTINGS TREATED WITH TALC DUSTS CONTAINING PLANT HORMONE ACIDS AND POTASSIUM SALTS

Data are means for four groups of eight cuttings

		Controls		Treated with talc containing 1000 p.p.m. of									Necessary difference, 5% level
				Acids					Potassium salts of				
		Un-treated	Talc treated	Indolyl-acetic	1-naphthyl-acetic	1-γ-naphthyl-butyric	1 and 2-γ-naphthyl-butyric	ε-(1-naphthyl)-hexoic	1-naphthyl-acetic	1-γ-naphthyl-butyric	1 and 2-γ-naphthyl-butyric	ε-(1-naphthyl)-hexoic	
Number of cuttings rooted	Transformed data	43.2	64.7	64.7	69.3	65.6	62.7	65.6	65.6	70.2	60.3	56.6	16.0
	Per cent	46.9	81.3	81.3	87.5	78.1	78.1	78.1	78.1	84.4	75.0	68.8	
Number of roots per rooted cutting		5.1	8.4	9.0	11.1	7.9	9.3	8.7	9.9	8.2	10.7	6.9	3.2
Length of root per rooted cutting, mm.		187	384	380	467	363	406	366	392	344	446	316	120
Mean root length, mm.		30.5	46.6	42.2	41.6	46.1	42.7	41.6	39.5	41.8	42.1	45.3	7.9
Number of cuttings with new growth	Transformed data	48.8	84.8	72.2	79.7	72.2	75.0	75.0	72.2	75.4	72.2	75.4	22.1
	Per cent	56.3	96.9	87.5	93.8	87.5	87.5	87.5	87.5	87.5	87.5	87.5	
Weight of new growth per cutting with new growth, gm.		0.08	0.22	0.19	0.25	0.23	0.19	0.22	0.22	0.22	0.25	0.18	0.09

of cuttings developing new growth. The weight of new growth per cutting with new growth was increased from two to three times by talc treatment. There were no other significant treatment effects. The beneficial effects of talc treatment thus stand out as the main feature of the results as a whole. There were no average differences between the groups of acids and salts, and the effects of the isomeric naphthylbutyric acids did not differ significantly from those of the pure butyric acid.

Deutzia scabra

The responses of *Deutzia* are presented in Table III. These cuttings rooted well and few treatments gave less than 90% rooting. The rooting of the talc control group just failed to exceed that of the untreated cuttings, but an increase was suggested. Hormone treatments, on the average, suggested a further increase in rooting, though none of the treatments differed significantly from the talc control in this respect when considered individually. Treatment with talc alone had no effect on the number of roots per rooted cutting of this species. Addition of naphthylacetic and the isomeric butyric acids resulted in more roots per rooted cutting than the number obtained with talc only, and all the salts, excepting those of the mixture of isomeric acids, yielded more roots than the talc control. An interesting reversal effect was noted. Treatment with salts of the isomeric butyric acids resulted in the production of fewer roots than did treatment with the acids, but the salt of hexoic acid produced more roots than the acid. The length of root mass was greater following application of the isomeric butyric acids than after the hexoic acid treatment. The latter reduced length of root mass below that of the talc control.

The number of cuttings showing new growth was, on the average, increased by talc treatment. None of the naphthyl treatments differed among themselves in this respect. Most of the naphthyl treatments resulted in lower weights of new growth than that following application of indolylacetic acid, which gave the greatest weight. Fresh root weight per group of eight cuttings appeared to be diminished following treatment with talc alone. However, a number of the naphthyl treatments resulted in weights exceeding those obtained following talc only. The isomeric butyric acids and the potassium salt of naphthylacetic exceeded the untreated control in respect to root weight, while the hexoic acid was below it. While there was no difference on the average between the results from acids and salts, certain differences between the chemicals were highly significant. Both acids and salts of the acetic and pure butyric members of the naphthyl series resulted in essentially the same weight of roots. Data for the weight of roots per group of eight cuttings and weight of root per rooted cutting indicated essentially the same type of reversal effect already discussed for the number of roots per rooted cutting. Further, talc treatment resulted in a lower weight of root per rooted cutting than occurred with the untreated group of cuttings.

TABLE III

RESPONSES OF DORMANT *Deutzia scabra* CUTTINGS TREATED WITH TALC DUSTS CONTAINING PLANT HORMONE ACIDS AND POTASSIUM SALTS

Data are means for four groups of eight cuttings

		Controls		Treated with talc containing 1000 p.p.m. of									Necessary difference, 5% level
				Acids					Potassium salts of				
		Un-treated	Talc treated	Indolyl-acetic	1-naphthyl-acetic	1-γ-naphthyl-butyric	1 and 2-γ-naphthyl-butyric	ε-(1-naphthyl)-hexoic	1-naphthyl-acetic	1-γ-naphthyl-butyric	1 and 2-γ-naphthyl-butyric	ε-(1-naphthyl)-hexoic	
Number of cuttings rooted	Transformed data	62.7	77.3	82.5	77.3	79.7	79.7	69.3	90.0	65.9	77.3	67.0	14.7
	Per cent	78.1	90.6	93.8	90.6	93.8	93.8	87.5	100.0	78.1	90.6	84.4	
Number of roots per rooted cutting		19.3	17.3	21.0	28.0	24.8	28.3	15.5	30.0	25.0	18.8	26.8	7.7
Length of root mass, mm.		66.0	57.3	56.8	52.5	56.3	62.5	45.0	51.0	62.5	58.3	58.3	12.0
Number of cuttings with new growth	Transformed data	58.6	72.2	84.8	69.8	79.7	67.9	62.3	73.1	67.9	67.9	79.7	18.5
	Per cent	71.9	87.5	96.9	84.4	93.8	81.3	78.1	84.4	81.3	81.3	93.8	
Weight of new growth per cutting with new growth, gm.		0.51	0.49	0.61	0.32	0.44	0.37	0.49	0.37	0.42	0.32	0.42	0.18
Fresh root weight, gm.		3.5	2.5	3.4	4.6	4.0	4.9	1.6	5.2	3.3	2.8	4.2	1.3
Average fresh root weight per rooted cutting, centigrams		55	36	45	64	53	67	23	65	56	39	61	19.0

The results, as a whole, indicate comparatively little effect from talc treatment. While there were no differences, on the average, between the response to naphthyl acids and salts taken as groups, there were marked differences between the behaviour of acids and salts of individuals. This difference was brought out by the drop in activity from the isomeric butyric acids to their salts and the increased activity of the hexoate as compared with hexoic acid.

Hydrangea arborescens

In Table IV are given data for the responses of *Hydrangea* cuttings. In the main, the responses of these cuttings disclosed treatment effects similar to those discussed in detail in the preceding three tables. Talc treatment had a marked beneficial effect on most of the responses. While there were no significant differences in the behaviour of the naphthyl acids or salts as groups, there were marked differences between the acids and salts of the last two members of the series. This reversal effect was closely similar to that discussed in detail for several of the responses of *Deutzia* cuttings (Table III). The isomeric mixture of butyric acids appeared rather more active than the corresponding mixture of salts, whereas naphthylhexoate was more active than the hexoic acid.

Iresine Lindeni

Data for the responses of *Iresine* cuttings are given in Table V. In general, treatment effects disclosed by the responses of these herbaceous cuttings were similar to those shown by dormant cuttings of the four woody plants. Beneficial effects of talc treatment were indicated, particularly in relation to reduced mortality. Injurious effects, as shown by increased mortality and reduced rooting, following treatment with 1000 p.p.m. indolylacetic acid, were however in contrast to the behaviour of the other plants considered. The number of roots per rooted cutting following naphthylacetic treatment was above that obtained with all other treatments to an extent not shown by any of the other plants. There were no differences between the groups of acids and salts but marked differences between the acids and salts of individual members of the naphthyl series in respect of length of root mass. The behaviour of the isomeric mixture of butyric acids and their salts and the hexoic acid and its salt was in agreement with the reversal effect mentioned in the case of *Deutzia* and *Hydrangea* cuttings, since physiological activity of the chemical appears to vary inversely with the length of root mass. Further, 1-γ-naphthylbutyric acid and its salt differed in respect of length of root mass. This was the only instance of any difference in effect of acid and salt of this member of the series. On the average, the naphthyl treatments resulted in a greater weight of root per rooted cutting than the untreated, talc or indolylacetic acid treated groups. Several of the naphthyl treatments were above indolylacetic acid in respect to root weight.

TABLE IV

RESPONSES OF DORMANT *Hydrangea arborescens* CUTTINGS TREATED WITH TALC DUSTS CONTAINING PLANT HORMONE ACIDS AND POTASSIUM SALTS

Data are means for four groups of eight cuttings

		Controls		Treated with talc containing 1000 p.p.m. of									Necessary difference, 5% level
				Acids					Potassium salts of				
		Un-treated	Talc treated	Indolyl-acetic	1-naphthyl-acetic	1-γ-naphthyl-butyric	1 and 2-γ-naphthyl-butyric	ε-(1-naphthyl)-hexoic	1-naphthyl-acetic	1-γ-naphthyl-butyric	1 and 2-γ-naphthyl-butyric	ε-(1-naphthyl)-hexoic	
Number of cuttings rooted	Transformed data	33.8	69.8	54.3	60.4	62.3	53.0	54.5	52.4	73.6	50.7	75.0	23.7
	Per cent	37.5	84.4	65.6	75.0	71.9	62.5	59.4	62.5	84.4	59.4	87.5	
Number of roots per rooted cutting		3.8	6.1	6.2	11.3	11.9	13.2	7.7	9.6	11.4	8.3	10.0	4.3
Length of roots per rooted cutting, mm.		81	144	130	191	200	244	139	138	229	140	184	81
Mean root length, mm.		15.8	24.4	21.8	17.4	17.0	19.1	19.0	14.4	19.7	16.4	18.6	7.4
Number of cuttings with new growth	Transformed data	43.4	72.2	52.2	59.9	66.6	54.8	63.2	52.4	73.6	52.4	72.2	23.1
	Per cent	53.1	87.5	62.5	68.8	78.1	65.6	68.8	62.5	84.4	62.5	87.5	
Weight of new growth per cutting with new growth, gm.		0.16	0.25	0.25	0.15	0.17	0.17	0.19	0.18	0.18	0.18	0.20	0.10
Fresh root weight, gm.		0.17	0.56	0.38	0.63	0.61	0.60	0.33	0.37	0.80	0.34	0.70	0.35

TABLE V

RESPONSES OF *Iresine Lindeni* CUTTINGS TREATED WITH TALC DUSTS CONTAINING PLANT HORMONE ACIDS AND POTASSIUM SALTS

Data are means for four groups of eight cuttings

		Controls		Treated with talc containing 1000 p.p.m. of									Necessary difference, 5% level
				Acids					Potassium salts of				
		Un-treated	Talc treated	Indolyl-acetic	1-naphthyl-acetic	1-γ-naphthyl-butyric	1 and 2-γ-naphthyl-butyric	ε-(1-naphthyl)-hexoic	1-naphthyl-acetic	1-γ-naphthyl-butyric	1 and 2-γ-naphthyl-butyric	ε-(1-naphthyl)-hexoic	
Number of cuttings dead	Transformed data	35.6	5.2	33.5	5.2	19.8	0	5.2	20.2	5.2	5.2	7.5	19.0
	Per cent	40.6	3.1	31.3	3.1	15.6	0	3.1	15.6	3.1	3.1	6.3	
Number of cuttings rooted	Transformed data	49.4	84.8	56.5	84.8	70.2	90.0	84.8	69.8	84.8	84.8	82.5	16.9
	Per cent	56.3	96.9	68.8	96.9	84.4	100.0	96.9	84.4	96.9	96.9	93.8	
Number of roots per rooted cutting		16.3	19.3	23.0	33.8	25.8	22.8	22.8	33.3	26.5	24.5	24.3	5.4
Length of root mass, mm.		22.3	21.5	17.5	14.8	24.3	22.5	26.5	16.3	20.5	23.3	22.8	3.7
Fresh root weight per rooted cutting, centigrams		1.7	1.9	1.4	1.6	2.2	2.3	2.3	2.0	2.3	2.2	2.0	0.8

Discussion

It is known that the higher members of the naphthyl series are active when used in solutions. The experiments just described show that when applied in a talc carrier, they compared favourably with naphthylacetic acid or its salt in respect to the number of cuttings rooted. This fact is of considerable interest since the effectiveness and scope of the dust carrier method of applying root growth promoting chemicals has been indicated by recent developments in vegetative propagation (3, 5, 8–15). Most of the other responses considered indicated that the higher members of the naphthyl series had physiological activity of the same order as naphthylacetic acid. On the whole, the activity of the isomeric mixture of butyric acids and the hexoic member of the series compared favourably with that of the other two members under test. However, the hexoic member of the series appeared, in several instances, to have somewhat less effect on the number and length of roots. This fact may be related to the use of identical weights of the various chemicals, since equal proportions in the dust provide fewer of the larger hexoic molecules.

In none of the five experiments was there any significant difference between the average effect on any response of the groups of naphthyl acids or salts. However, in several experiments there were substantial differences between the acids and salts of the isomeric butyric and the hexoic members of the naphthyl series. There was a tendency for the isomeric acids and the potassium salt of naphthylhexoic acid to be more active than the corresponding mixture of salts or the hexoic acid. It would appear justifiable to attribute this behaviour of the isomeric mixture to the presence of 2-γ-naphthylbutyric acid. The reduced activity of its salt would seem to be related to greater solubility or transport considerations. The activity of potassium naphthylhexoate would also appear to be related to solubility or transport considerations. These results would not suggest that salts of growth stimulating acids are always to be preferred to the acids (16).

Reference may be made to one point that has frequently been stressed by the author's experiments on propagation. This relates to the variability in different parts of the propagation frame which, in conjunction with the natural biological variability, renders it most important to plan experiments in a manner that will permit of some test of significance of the results. A case in point is noted in Table I. The weight of new growth per cutting with new growth varied from a low of 0.28 gm. after naphthylacetic acid treatment, to a high of 0.55 gm. after application of potassium 1-γ-naphthylybutyrate. Despite this approximately 100% difference in weight, statistical analysis showed that the variability of the data was too great to demonstrate any significant effects. In consequence any attempt to discern treatment effects from the means of single small groups of cuttings without replication seems a most dubious procedure.

A rather striking feature of the results was the beneficial effect of the talc treatment. Beneficial effects from treatment with dusts have been recognized

previously (5, 6, 11, 12, 14, 15). Not only root development but even the initiation of new growth by dormant cuttings was substantially increased by talc treatment. It appears likely that water relations do account for a considerable part of the beneficial effect (11). However, it is also probable that availability of certain mineral elements may be involved, since nutrient treatments have been shown to affect the rooting of cuttings (2, 6, 10, 15). This possibility would seem to be indicated by data in Tables II and IV, which show that talc treatment increased the weight of new growth per cutting with new growth. There is, of course, the possibility that an adsorbent such as talc could retain natural plant hormones that might be leached from the open end of the cuttings. Complete elucidation of the mechanism of the action of talc on cuttings awaits further experimental study. However, the facts available to date indicate that the carrier dust method of treating plant cuttings has numerous advantageous features.

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THE GEOGRAPHICAL DISTRIBUTION OF THE GENUS *GYMNOSPORANGIUM*[1]

By Ivan H. Crowell[2]

Abstract

This study of geographical distribution shows that species of the genus *Gymnosporangium* are found in the northern hemisphere only and occur most abundantly in the temperate portion. Each of the three major continents contains a distinctive *Gymnosporangium* flora and, with the exception of three species called the "tricontinental species", species occur naturally in one continent only. The genus contains about 48 species; 33 occur in North America, 15 in Asia and 6 in Europe (including the three tricontinental species in each case). Explanations of the types of geographical distribution of the North American species are given under four categories: (i) species that occupy all potential territory covered by the coincident ranges of their alternate hosts, (ii) species that are confined by the range of their "primary" telial host, (iii) localized species that are confined within a portion of the coincident ranges of their alternate host, and (iv) widely distributed species that are not limited in their range by either alternate host group.

Introduction

The phytopathological and mycological literature is replete with incidental statements on the geographical distribution of fungi, yet few authors have dealt comprehensively with this subject. In contrast to the geographical distribution of higher plants, that of fungi is limited by the distribution of their living or dead substrata. Such limitations are particularly true of parasitic fungi that can exist on living substrata only. Of especial interest is the geographical distribution of opsis-form rusts, such as species of *Gymnosporangium*, for these obligate parasites, lacking the uredial or repeating-spore stage, have no means of perpetuating themselves beyond the coinciding ranges of the two groups of alternate host plants. It might be expected that species of *Gymnosporangium* would occupy completely territory covered by the coinciding ranges of their alternate hosts. Such, however, is seldom the case.

The genus *Gymnosporangium* is composed of about 48 species and is confined almost entirely to the temperate portion of the northern hemisphere. This region, especially North America and Europe, is probably more intensively explored and its microflora more thoroughly known than is the case with any other. Since the fruiting structures of species of *Gymnosporangium* are quite conspicuous, it seems fair to assume that the numerous records in the literature and on collections deposited in herbaria afford comprehensive (though obviously incomplete) material on which to base geographical studies.

The present account of the geographical distribution of the genus *Gymnosporangium* is offered after the writer's examination, during the past seven years, of approximately three thousand records.

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The pertinent literature of North America, Asia, and Europe was carefully read and notes were taken of reports of the occurrence of all species of the genus *Gymnosporangium* on all hosts. These notes were augmented by examination of specimens from several herbaria in the United States, Canada, and Europe. Furthermore, several co-operators sent lists of the species of *Gymnosporangium* in their herbaria. From these notes and the writer's own observations, host lists were compiled for each species. Maps were drawn showing the station for each report of the rust wherever possible—a dot representing the station. Superimposed on these maps were representations of the geographical ranges of *(a)* the O–I hosts indicated by vertical lines, and *(b)* the III hosts, indicated by horizontal lines. Rehder's Manual (1927) was the principal source for information on taxonomy and distribution of hosts, but several other taxonomic works were also consulted.

Upon analysing the maps of distribution of the species of *Gymnosporangium* it was found that three major geographical regions were clearly discernible. These geographical regions were contained one within each of the continents of North America, Asia, and Europe (including the adjacent portion of northern Africa). In the North American region, two minor geographical areas were also evident. These were *(a)* the eastern region, extending from the Atlantic ocean to the foothills of the Rocky Mountains, and *(b)* the western region, extending from the foothills of the Rocky Mountains westward to the Pacific Coast.

Three species of *Gymnosporangium* occur on all continents of the northern hemisphere and hence did not find a definite place in the discussion of species of any one region. The three species are dealt with separately as the "Tricontinental Species". Discussion of the geographical distribution of other species of *Gymnosporangium* are presented according to the scheme of major and minor geographical areas.

The Tricontinental Species

Three species of *Gymnosporangium* are found throughout the northern hemisphere. They are *(a) G. aurantiacum* Chev., *(b) G. clavariaeforme* (Jacq.) DC., [*G. gracile* Pat., *G. orientale* Syd., *G. oxycedri* Bres.], and *(c) G. juniperinum* (L.) Mart., [*G. amelanchieris* Fisch., *G. ariae-tremelloides* (A. Br.) v. Tub.]. For the purposes of clarity in this account, marginal or inadequately differentiated species have been included as synonyms of the three broadly conceived species *G. aurantiacum*, *G. clavariaeforme*, and *G. juniperinum*. This procedure follows closely that of Eriksson (8). Recognition of forms of doubtful specific rank would have necessitated treatment of small, closely related entities, most of which seem to be of local distribution on a few hosts.

The tricontinental species have several characteristics in common apart from their wide distribution. They are most abundant in and most closely related to the European *Gymnosporangium* flora. Their principal telial hosts are species of *Juniperus* in the section Oxycedrus, chiefly *J. communis*.

Gymnosporangium clavariaeforme and *G. juniperinum* have been reported to attack species in the section Sabina, but they do not seem to do so frequently.

Each of the tricontinental species occurs over the greater part of Europe and adjacent north Africa. In other geographical regions each species is less abundant and stations are more widely scattered, though hosts for them seem to have an unbroken distribution throughout the greater part of the temperate portion of the northern hemisphere.

Details of the geographical distribution of these species are shown in Figs. 1, 2, and 3 respectively. Stations for the three tricontinental species are brought together in Fig. 4. Their distribution in Europe is essentially like that of strictly European species. Their distribution in North America is scattered and broken. The conspicuous gap in distribution from Europe to coastal Asia probably represents a lack of knowledge of the fungi of this vast, little explored region rather than an absence of these species of *Gymnosporangium*.

Species of the Eastern North American Region

A. Endemic Species—

1. *G. bermudianum*	6. *G. ellisii*	11. *G. hyalinum*
2. *G. biseptatum*	7. *G. exiguum*	12. *G. juniperi-virginianae*
3. *G. clavipes*	8. *G. externum*	13. *G. nidus-avis*
4. *G. corniculans*	9. *G. floriforme*	14. *G. trachysorum*
5. *G. davisii*	10. *G. globosum*	15. *G. transformans*

B. Tricontinental Species—

16. *G. aurantiacum*	17. *G. clavariaeforme*

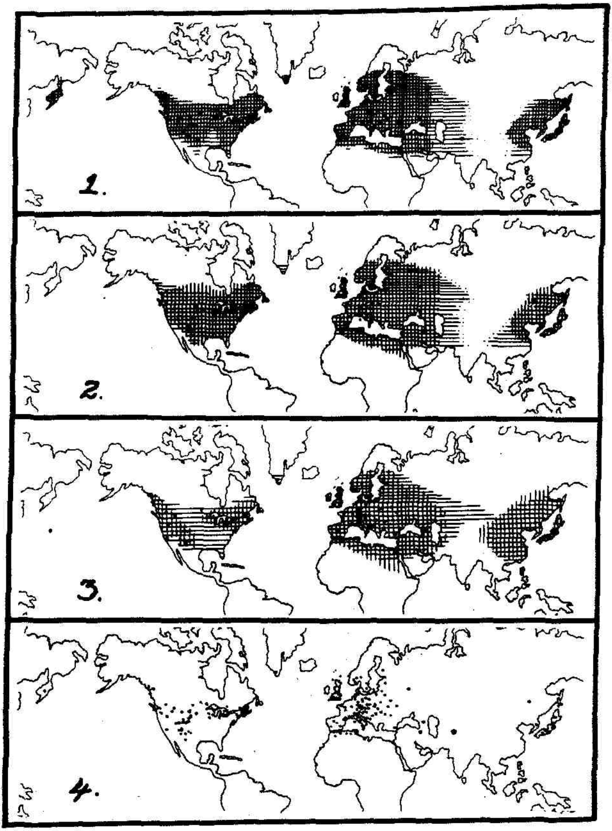
C. Exotic Species—

18. *G. haraeanum*	19. *G. japonicum*

The eastern North American region contains one of the richest and most diversified *Gymnosporangium* flora of the world. This wealth of material is significant in several respects. Nineteen of the 48 species discussed are recorded from this region; of these, 15 are endemic, 2 are exotic (native to Asia), and 2 of the tricontinental species are considered to be indigenous.

The greatest concentration of species of *Gymnosporangium* is found along the Atlantic coastal area of the eastern region, and the abundance of stations diminishes toward its western boundary. With the exception of a few stations for *G. clavipes* and *G. juniperi-virginianae* in the western region of North America, all other endemic species are strictly localized within the eastern region, none of them ranging over the maximum territory occupied by the coincident ranges of their alternate hosts.

Four species are generally distributed in eastern North America. They are *G. juniperi-virginianae* Schw., *G. clavipes* C. & P., *G. globosum* Farl., and



FIGS. 1 – 4. *The geographical distribution of Gymnosporangium in relation to that of alternate host groups. Vertical lines, aecial hosts; horizontal lines, telial hosts; dots, stations for the rust.* FIG. 1. *G. aurantiacum.* FIG. 2. *G. clavariaeforme.* FIG. 3. *G. juniperinum.* FIG. 4. *The tricontinental species.*

G. nidus-avis Thax.* (Figs. 5, 6, 7, and 8.) It will be seen that although their distribution is wide, none of these species occupies all potential territory; *G. juniperi-virginianae* seems to make the nearest approach to this maximum, but a consideration of the following will show this to be far from true. Pomaceous hosts for this species include all native species and many orchard varieties of *Malus*. The combined ranges of these plants is essentially transcontinental in temperate North America. So also are the combined ranges of the telial hosts *Juniperus virginiana* L., *J. scopulorum* Sarg., and *J. horizontalis* Moench. The fungus, however, is confined almost entirely within coincidental ranges of native species of *Malus* and *J. virginiana* in eastern North America.

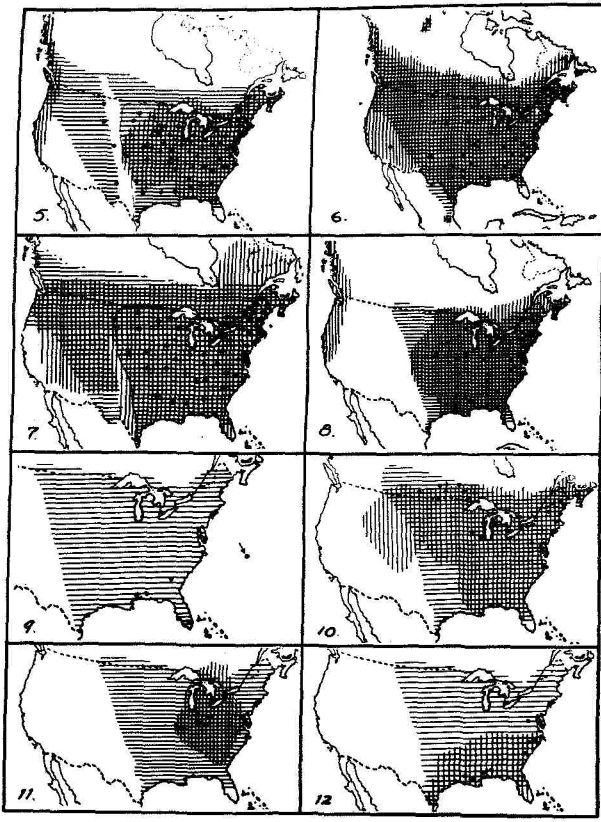
In Fig. 5 are shown four stations in the Rocky Mountains beyond the range just mentioned. These stations occurred on *J. virginiana* and *J. scopulorum*. In two stations in New Mexico that were checked by the author, the rust was found on hosts imported for ornamental purposes. No evidence of the rust could be found after the galls produced one crop of spores.

G. clavipes is a species of exceptional interest to the problem at hand because of its great longitudinal and latitudinal range. The species extends from Lake Athabasca, Alberta, to Mexico City, Mexico, and from the Atlantic Ocean almost to the Pacific. *G. clavipes* is the only species to parasitize consistently telial hosts in two sections of the genus *Juniperus*, in particular *J. virginiana* in the section Sabina and *J. communis* in the section Oxycedrus. Its pomaceous and *Juniperus* hosts are associated over the greater part of the northern hemisphere, yet this species is confined to North America and is predominately an eastern species. As stations for *G. clavipes* are being recorded from time to time in the western region, it appears that it may be actively extending its geographical range, a phenomenon not in evidence in the case of any other species of the genus.

Though alternate hosts for *G. globosum* are transcontinental in range, the rust is found in eastern North America almost entirely within the range of the telial host *J. virginiana*. With the exception of *Malus fusca* all other endemic hosts of *G. nidus-avis* extend over much of the area occupied by *J. virginiana*.

The four species discussed in the foregoing are probably the most intensively studied in the genus. In contrast to the wide distribution of these species, certain others are conspicuously limited in their geographical range. *G. bermudianum* (Farl.) Earle (Fig. 9) is an outstanding example. It is an autoecious species and hence has a potential range as great as the combined ranges of its hosts—*J. virginiana* L. and *J. barbadensis* L. The rust has been reported from relatively few widely separated stations in Bermuda and the Bahamas Islands and from a narrow strip of the mainland bordering the Gulf of Mexico. One station, more inland, is located in Georgia.

* *On the basis of numerous cultural experiments and microscopic examinations, the author considers G. nidus-avis Thaxter (Bull. Conn. Exp. Sta. 107 : 3, 1891) and G. effusum Kern (Bull. N.Y. Bot. Gard. 7 : 459, 1911) to be synonymous.*



FIGS. 5 – 12. *The geographical distribution of Gymnosporangium in relation to that of alternate host groups. Vertical lines, aecial hosts; horizontal lines, telial hosts; dots, stations for the rust.* FIG. 5. *G. juniperi-virginianae.* FIG. 6. *G. clavipes.* FIG. 7. *G. globosum.* FIG. 8. *G. nidus-avis.* FIG. 9. *G. bermudianum.* FIG. 10. *G. corniculans.* FIG. 11. *G. externum.* FIG. 12. *G. trachysorum.*

G. corniculans Kern, *G. externum* Arth. & Kern, and *G. trachysorum* Kern (Figs. 10, 11, and 12) are examples of species that are distributed in a narrow zone extending east and west across the eastern region. *G. corniculans* is found only in the northern extreme of the coincident ranges of its alternate hosts. *G. externum* has been reported from a narrow corridor that runs through the middle portion of the coincident range of its alternate hosts. *G. trachysorum* is a little known species reported from a few stations near the Atlantic Coast and the Gulf of Mexico in the United States.

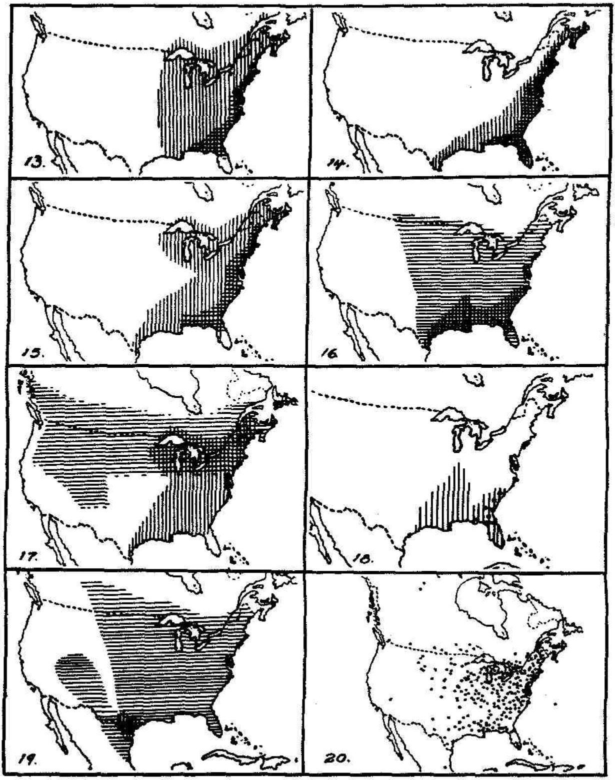
In contrast to the east and west distribution of the three species just discussed is the north and south distribution of three species whose telia occur on *Chamaecyparis thyoides*, namely, *G. biseptatum* Ellis, *G. ellisii* (Berk.) Farl., and *G. transformans* (Ellis) Kern (Figs. 13, 14, and 15). With the exception of the single inland station in the vicinity of Lake Ontario in New York, all other stations for these species are along the Atlantic Coast. Each of these species is most abundant in the northern part of its host's ranges, and it would seem that they are host-limited in this region. Between the Delaware peninsula and the Gulf of Mexico is an extensive area in which both kinds of alternate hosts are reported. *G. transformans* has not been reported from this area, but *G. ellisii* and *G. biseptatum* are found in the most southern portion of it, approximately one thousand miles south of the nearest northern station.

Three species of *Gymnosporangium* are definitely limited by the ranges of their aecial hosts. These are *G. floriforme* Thax. of the southern portion, *G. davisii* Kern of the northern portion, and *G. exiguum* Kern, intermediate between the eastern and western regions (Figs. 16, 17, and 19). Though fully as conspicuous as most other species, particularly on their aecial hosts, the few collections of these species indicate that they are among the rarest in eastern North America.

In eastern North America one species only, namely, *G. hyalinum* (Fig. 18), is as yet unconnected with a telial host. Its aecial hosts are species of *Crataegus*.

Two exotic species have been found in eastern North America. These are *G. haraeanum* Syd. and *G. japonicum* Syd. (Figs. 37 and 38), both native to eastern Asia. They were found on imported ornamental junipers most of which were destroyed shortly after examination at the port of entry. As the species have not been reported since, it is assumed that they did not become established. They did, however, become established on the Pacific Coast of western North America.

To complete the discussion of species of *Gymnosporangium* in eastern North America, it should be recalled that two of the tricontinental species, namely, *G. aurantiacum* and *G. clavariaeforme*, have been recorded from the region. Stations for species of *Gymnosporangium* of the eastern region have been plotted together in Fig. 20.



FIGS. 13 – 20. *The geographical distribution of Gymnosporangium in relation to that of alternate host groups. Vertical lines, aecial hosts; horizontal lines, telial hosts; dots, stations for the rust.* FIG. 13. *G. biseptatum.* FIG. 14. *G. ellisii.* FIG. 15. *G. transformans.* FIG. 16. *G. floriforme.* FIG. 17. *G. davisii.* FIG. 18. *G. hyalinum.* FIG. 19. *G. exiguum.* FIG. 20. *Gymnosporangia of the eastern North American region.*

Species of the Western North American Region

A. Endemic species—

1. *G. betheli*	6. *G. juvenescens*	11. *G. nelsoni*
2. *G. cupressi*	7. *G. kernianum*	12. *G. nootkatense*
3. *G. guatemalianum*	8. *G. libocedri*	13. *G. speciosum*
4. *G. harknessianum*	9. *G. meridissimum*	14. *G. tubulatum*
5. *G. inconspicuum*	10. *G. multiporum*	15. *G. vauqueliniae*

B. Tricontinental species—

16. *G. aurantiacum*	17. *G. clavariaeforme*	18. *G. juniperinum*

C. Exotic species—

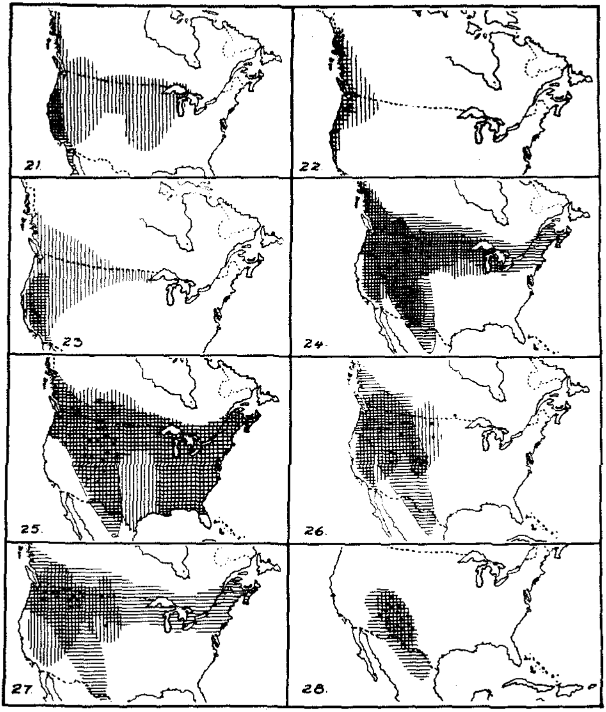
19. *G. haraeanum*		20. *G. japonicum*

This region vies with the eastern region in the richness and diversification of its *Gymnosporangium* flora. Twenty species are reported from the western region. These are made up of 15 endemic species, 2 exotic species (the same 2 from Asia that were found in the eastern region), and the 3 indigenous tricontinental species. Geographically the species are distributed in two distinct sub-regions corresponding to the two major mountain chains. Thirteen of the species (10 endemic and 3 tricontinental) are found in the Rocky Mountains while 5 (3 endemic and 2 exotic) are found in the Sierra Nevada and Cascade Mountains, and 2 species have recently been found in Guatemala. No species of *Gymnosporangium* is generally distributed through the whole of the western region, but the more widely distributed species occur in both mountain ranges, where the latter merge in the northern United States and southern Canada. It is interesting to observe that several species are found over the maximum available territory covered by the coincident ranges of their alternate hosts—a phenomenon not found elsewhere for this genus of rusts.

The species of *Gymnosporangium* in western North America will now be discussed in detail—first, those that occur in the Cascade and Sierra Nevada Ranges. *G. libocedri* (P. Henn.) Kern (Fig. 21) is one of the species that occupies all potential territory covered by the coincident ranges of its alternate hosts. Although few stations are recorded for *G. nootkatense* (Trel.) Arth. (Fig. 22), it also probably occupies all potential territory. *G. harknessianum* (E. & E.) Kern (Fig. 23) extends over a small portion of its potential territory.

Two exotic Asiatic species, *G. haraeanum* and *G. japonicum* (Figs. 37 and 38) seem to have become established along the Pacific coast parasitizing, however, exotic hosts only.

In the larger sub-region of western North America, the Rocky Mountain region, three species of *Gymnosporangium* are rather widely distributed from southern Alaska to New Mexico. *G. nelsoni* Arth. (Fig. 24) is the most widely distributed and abundantly represented species in the western region. Its alternate hosts, however, extend far beyond the range of the rust. *G. juvenescens* Kern (Fig. 25) is also generally distributed in the Rocky Mountains.



FIGS. 21 – 28. *The geographical distribution of Gymnosporangium in relation to that of alternate host groups. Vertical lines, aecial hosts; horizontal lines, telial hosts; dots, stations for the rust.* FIG. 21. *G. libocedri.* FIG. 22. *G. nootkatense.* FIG. 23. *G. harknessianum.* FIG. 24. *G. nelsoni.* FIG. 25. *G. juvenescens.* FIG. 26. *G. betheli.* FIG. 27. *G. tubulatum.* FIG. 28. *G. speciosum.*

Of rather scattered distribution is *G. betheli* Kern (Fig. 26). Its stations tend to be clustered together in small groups, indicative of localized centres for the rust. This may be explained in part by the fact that certain of its aecial hosts—species of *Crataegus*—are said to prevail chiefly near lakes and streams.

All other endemic species of *Gymnosporangium* in the western region are of local distribution. One of these, *G. tubulatum* Kern (Fig. 27) is confined

to the more northerly portion of the coincident ranges of its alternate hosts. Three species, *G. speciosum* Peck, *G. kernianum* Bethel, and *G. inconspicuum* Kern (Figs. 28, 29, and 30) are found over all potential territory. *G. speciosum* is one of the most abundantly represented species in the southern portion of the Rocky Mountains. It doubtless extends southward into Mexico, yet no station is recorded for it there. *G. kernianum* is found over essentially the same area as *G. speciosum*, though it appears to be less common. *G. inconspicuum*, like the two species just discussed, is limited to the southern portion of the Rocky Mountains. It occupies all potential territory.

Four species in western North America are as yet unconnected with alternate hosts. *G. cupressi* Long and Goodding (Fig. 31) is probably of limited distribution, since its telial host *Cupressus arizonica* Greene is confined to southwestern Arizona. *G. meridissimum* Crowell and *G. guatemalianum* Crowell (also Fig. 31) are known from a few stations in Guatemala. Since no other species of *Gymnosporangium* is known from Guatemala, these may represent a single species. *G. multiporum* Kern (Fig. 32) though probably limited to the southern Rocky Mountains may be of wider distribution. *G. vauqueliniae* Long & Goodding (also Fig. 31) is known from the type locality only in southern Arizona. It will be recalled that the tricontinental species are scattered through the Rocky Mountains, and in the northern portions are found generally over the western region. Stations for endemic species of *Gymnosporangium* in western North America, except the Guatemalian ones, are shown in Fig. 33.

Species of the Asiatic Region

A. Endemic species—

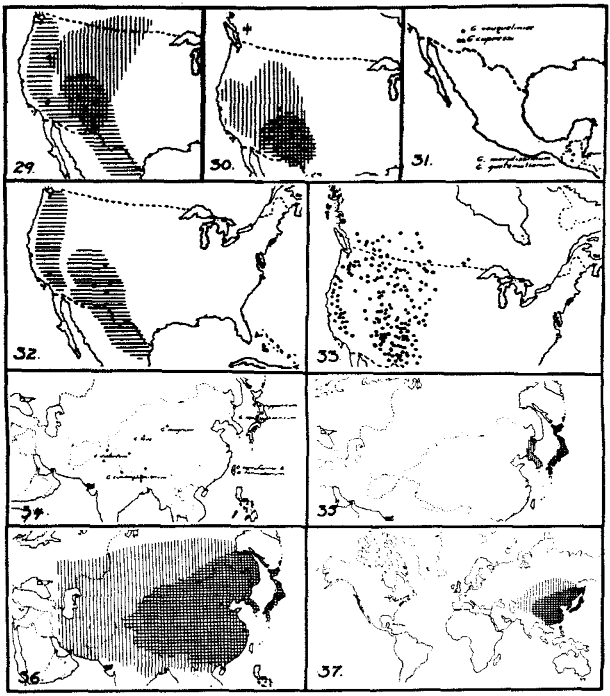
1. *G. corniforme*	5. *G. haraeanum*	9. *G. magnum*
2. *G. cunninghamianum*	6. *G. hemisphaericum*	10. *G. miyabei*
3. *G. distortum*	7. *G. japonicum*	11. *G. nipponicum*
4. *G. formosanum*	8. *G. leve*	12. *G. yamadae*

B. Tricontinental species—

13. *G. aurantiacum*	14. *G. clavariaeforme*	15. *G. juniperinum*

The mycological flora of Asia is probably less known than that of other geographical regions, yet the *Gymnosporangium* flora, though sparsely represented in number of collections, is so abundant in number of species in widely separated stations as to indicate a richness that may rival that of North America.

Fifteen species of *Gymnosporangium* are reported from Asia. Twelve are endemic and three are indigenous. Eight of the endemic species are known from the type locality only or from a few stations in the vicinity. These species are *G. cunninghamianum* Barcl. in northeastern India and southern Tibet (6 stations recorded, 3 only could be plotted); *G. distortum* Arth. & Cummins from northern India and western China (4 stations, 3 plotted); *G. leve* Crowell and *G. magnum* Crowell known from their type localities in



FIGS. 29 – 37. *The geographical distribution of Gymnosporangium in relation to that of alternate host groups. Vertical lines, aecial hosts; horizontal lines, telial hosts; dots, stations for rust.* FIG. 29. *G. kernianum.* FIG. 30. *G. inconspicuum.* FIG. 31. *G. cupressi. G. guatemalianum, G. vauqueliniae, G. meridissimum.* FIG. 32. *G. multiporum.* FIG. 33. *Gymnosporangia of the western North American region. (Guatemalian stations not shown, see Fig. 31.)* FIG. 34. *Shows stations for 8 species of Gymnosporangium in Asia.* FIG. 35. *G. miyabei.* FIG. 36. *G. yamadae.* FIG. 37. *G. haraeanum.*

China; *G. nipponicum* Yamada (type locality); *G. hemisphaericum* Hara (2 stations, both plotted) of Honshu, Japan, and *G. corniforme* Sawada and *G. formosanum* Hirat. known from their type localities in Formosa. Stations for these eight species have been plotted on Fig. 34.

Two endemic species are of wide distribution in Japan, *G. miyabei* Yamada & Miyake and *G. yamadae* Miyabe (Figs. 35 and 36).

Two species, seemingly endemic to Asia, have been imported to both North America and Europe; these are *G. haraeanum* Syd. and *G. japonicum* Syd. (Figs. 37 and 38). Both species are found in Japan and coastal China. Of much interest was the discovery of these species on junipers imported in Connecticut about 1910. The fungi have not been reported from eastern North America since. In western North America, these rusts have become established on Asiatic hosts and were probably imported on one or both alternate hosts. Recently these species have been reported from France; *G. haraeanum* was reported on *J. chinensis* and *G. japonicum* on *Pyrus sinensis* Lindl.

The three indigenous tricontinental species have been collected at several stations in Japan. Each of the species attacks endemic and indigenous hosts; this would seem to indicate that they occur naturally in Asia rather than being introduced. Stations for the 12 endemic Asiatic species are plotted in Fig. 39. The concentration of stations in Japan and the west coast of Asia is probably due to the more intensive mycological studies carried on there.

Species of the European Region

A. Endemic Species—

1. *G. confusum* 2. *G. minus* 3. *G. sabinae*

B. Tricontinental Species—

4. *G. aurantiacum* 5. *G. clavariaeforme* 6. *G. juniperinum*

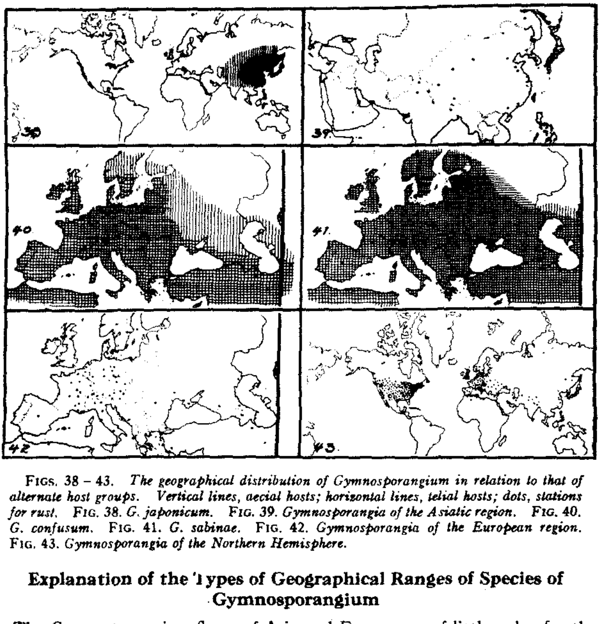
C. Exotic Species—

7. *G. globosum* 8. *G. haraeanum* 9. *G. japonicum*

This region, including north Africa, though the smallest of the geographical regions, contains a disproportionally meager *Gymnosporangium* flora. Nine species occur in Europe. Of these, three have been introduced, but are probably not established—*G. globosum*, *G. japonicum*, and *G. haraeanum*. Each of the three tricontinental species, *G. aurantiacum*, *G. clavariaeforme*, and *G. juniperinum* are generally distributed over much of the European region. The recently described species *G. minus* Crowell on *Cupressus sempervirens* L. is known from the type locality in Athens, Greece.

Thus, of the nine species reported from Europe two only, *G. confusum* Plowr. and *G. sabinae* (Dicks.) Wint. [G. *fuscum* Hedw. F., *G. tauricum* J. Ericks.] (Figs. 40 and 41) are endemic and generally distributed in Europe. Each is doubtless present in greater abundance than represented on the maps. Scores of records are at hand that cannot be plotted because of insufficient information.

In Fig. 42, stations for the three endemic European species, *G. confusum*, *G. sabinae*, and *G. minus* are plotted. An examination of this map will show that they are distributed throughout much of central Europe. The scattering of stations eastward to the Black and Caspian Seas seems to indicate a still farther extension into Asia.



FIGS. 38 – 43. *The geographical distribution of Gymnosporangium in relation to that of alternate host groups. Vertical lines, aecial hosts; horizontal lines, telial hosts; dots, stations for rust.* FIG. 38. *G. japonicum.* FIG. 39. *Gymnosporangia of the Asiatic region.* FIG. 40. *G. confusum.* FIG. 41. *G. sabinae.* FIG. 42. *Gymnosporangia of the European region.* FIG. 43. *Gymnosporangia of the Northern Hemisphere.*

Explanation of the Types of Geographical Ranges of Species of Gymnosporangium

The *Gymnosporangium* floras of Asia and Europe are of little value for the purposes at hand, since in the former region the flora is scantily represented and in the latter the few widely distributed and one localized species offers little variation for discussion. The *Gymnosporangium* flora of North America composed of several species of wide geographical ranges affords excellent opportunities for observation and comparisons. The types of geographical ranges for species of *Gymnosporangium* may be classified in four categories as follows:—

(*A*) Species that occupy all potential territory covered by the coincident ranges of their alternate hosts.

(*B*) Species that occupy less than all potential territory.

1. Species that are confined by the range of their "primary" telial host.
2. Localized species that are confined within a portion of the coincident ranges of their alternate hosts.

3. Widely distributed species that are not limited in their range by either alternate host group.

These categories will be discussed in the order of their arrangement in the key.

(A) Species that Occupy All Potential Territory Covered by the Coincident Ranges of Their Alternate Hosts

Species in Category *(A)* are found in Western North America only and include *G. libocedri*, *G. nootkatense*, *G. inconspicuum*, *G. kernianum*, and *G. speciosum* (Figs. 21, 22, 28, 29, and 30). These species are definitely and sharply limited by the coinciding ranges of their alternate hosts. With the exception of the eu-form species *G. nootkatense*, they have no means of perpetuating themselves beyond the coinciding ranges of their alternate hosts. *G. nootkatense* occurs over the full extent of its II-III host and hence occupies all potential territory.

(B) Species that Occupy Less than All Potential Territory

1. Species that are confined by the range of their "primary" telial host.

The phrase "primary telial host" requires explanation. The writer's unpublished study of hosts of the genus *Gymnosporangium* has revealed that one species of *Juniperus* in each geographical region had all or most of the following characteristics: *(a)* it appeared to be the most susceptible host, *(b)* it was attacked by most or all the species of *Gymnosporangium* parasitizing hosts in the section Sabina of the genus *Juniperus*; *(c)* it was the most widely distributed species. In eastern North America, *Juniperus virginiana* was the most susceptible telial host; it was attacked by all the species of *Gymnosporangium* parasitizing hosts in the section Sabina; it occurred over the greater portion of the eastern region. These statements are largely true of *J. scopulorum* in western North America. *J. chinensis* appears to be the primary telial host in Asia. A primary telial host can hardly be said to exist for the few species of *Gymnosporangium* in Europe. Other telial hosts may be called "supplementary" telial hosts, and are characterized by being more resistant, by being attacked by fewer species of *Gymnosporangium*, and by being of more local distribution. Not all species of these rusts have supplementary telial hosts, for many are known to occur on the primary telial hosts only.

Six species are placed in Category (1)—*G. juniperi-virginianae*, *G. globosum*, and probably *G. nidus-avis* of eastern North America; *G. nelsoni*, *G. juvenescens*, and *G. betheli* of western North America (Figs 5, 7, 8, 24, 25, and 26). In order that a rust maintain itself in numerical equilibrium over a period of years, one infection must produce another. An essential requirement in this genus appears to be the high susceptibility of the primary telial host. An illustration of this phenomenon may be taken from the reaction of *Juniperus* sp. to infection by *G. juniperi-virginianae*. In nurseries in Massachusetts where various species of junipers grow in large numbers close together it was repeat-

edly observed that the primary telial host, *Juniperus virginiana*, invariably supported the preponderance of infections of *G. juniperi-virginianae*. While other junipers would be attacked, one often had to search for the isolated infections. Such resistance of supplementary telial hosts appears to prevent the rust from perpetuating itself over territory occupied by them, but in which the primary telial host is not found. An examination of the maps of distribution of the species in Category (1) will show that the ranges of the rusts coincide closely with the ranges of the primary telial hosts—which are outlined on the maps by means of a broken line. A minor exception should be noted for *G. betheli* (Fig. 26), whose aecial hosts do not extend into the southern portion of the range of the primary telial host, though the rust extends as far south as the aecial hosts permit.

According to the theory it is unlikely that *G. juniperi-virginianae*, the cedar-apple rust fungus, will extend into such apple growing regions as the Maritime Provinces or the Pacific States where, although alternate hosts are present in abundance, the primary telial host is absent. The theory also offers an explanation as to how *G. japonicum* and *G. haraeanum* of Asia became established on the Pacific Coast of North America—their primary telial host, *J. chinensis*, (and aecial hosts) were imported and became established earlier than the rusts or at the same time.

2. *Localized species that are confined within a portion of the coincident ranges of their alternate hosts.*

In Category (2) are placed seven exemplary species—*G. bermudianum*, *G. corniculans*, *G. externum*, *G. trachysorum*, *G. floriforme*, *G. davisii*, and *G. tubulatum* (Figs. 9, 10, 11, 12, 16, 17, and 27). It will be observed that each of these species has its maximum range in an east and west direction. This is also the direction of isothermic lines of spring temperatures in the region of distribution of these rusts. Hence temperature is probably a potent factor influencing the range of these species. That temperature alone probably does not restrict the geographical ranges of these species of *Gymnosporangium* is indicated by the finding that the temperature range for sporulation of most species is from 3° to 30° C.—a range wider than that which usually occurs during the spring weeks, when teliospores germinate, and the summer and fall months, when aeciospores probably germinate. That humidity alone probably does not restrict the geographical ranges of these species of *Gymnosporangium* is indicated by the fact that rains regularly occur during the critical sporulating periods. As they are remarkably resistant spores, the time over which favourable temperatures and humidity conditions can occur can be of several weeks duration. It has been observed by several authors that teliospores may be mature and a high percentage of them germinate well in advance of production of new, susceptible growth by aecial hosts. However, as most species of *Gymnosporangium* are perennial on their telial hosts, should favourable conditions fail to occur in one year, the rust can abide the fortunes of one and probably more subsequent years.

It has been shown that aecial hosts of *G. globosum*, *G. juniperi-virginianae*, and *G. clavipes* (4, 5, 12) have a definite period during which they can become infected. This period of susceptibility usually extends from a few days to a few weeks after growth begins in the spring. That a period of susceptibility exists for hosts of any species in Category (2) is not definitely known, but one is presumed to exist for each aecial host. It has been observed that the period of susceptibility of some aecial hosts is passed during the spring, when humidity conditions did not permit infection to occur. These phenomena of temperature, spore germination, precipitation, and period of susceptibility of aecial hosts in the spring materially lessen the possibilities of infection by species of *Gymnosporangium*. Based on these phenomena, it is suggested that the narrow geographical range of species of *Gymnosporangium* in Category (2) represents the zone over which favourable environmental conditions consistently occur during the period of susceptibility of the aecial hosts as the spring season advances northward.

Four additional species of *Gymnosporangium*—*G. biseptatum*, *G. ellisii* and *G. transformans* of eastern North America and *G. harknessianum* of the western region (Figs. 13, 14, 15, and 23) probably belong in this category or are a distinct group. Their distribution differs from that of the others in that the single telial host of each species seems to be the limiting host but not the limiting factor in their distribution. The first three species are definitely telial-host limited in the northern portion of their range. Very disturbing are the stations for *G. biseptatum* and *G. ellisii* in the southern portion of eastern North America, approximately 1000 miles away from the nearest station for the rust. It seems unwise, with the meagre data available, to hazard an explanation of the geographical distribution of these four species.

Several species of *Gymnosporangium* as *G. nelsoni*, *G. juvenescens*, *G. betheli*, *G. kernianum*, and *G. inconspicuum* (Figs. 24, 25, 26, 29, and 30) are reported to be found at stations far removed from the main centres. Some of the collections have been carefully checked. How they occurred does not seem to be clear, for none of these species can maintain itself on one host. Could they be due to the chance finding of sporadic infections resulting from wind, bird, or insect carried spores? It would be interesting, indeed, to have accurate data on the duration and extent of the infections in such isolated places. Some of the isolated stations for certain species are subject to question, for it is difficult, if not impossible, to identify certain species of *Gymnosporangium* from the aecial or telial phase alone.

3. *Widely distributed species that are not limited in their range by either alternate host group.*

Some species of *Gymnosporangium* in Category (3), namely, *G. aurantiacum*, *G. clavariaeforme*, and *G. juniperinum* (Figs. 1, 2, and 3) seem to owe their wide distribution in large measure to the facts that the telial host, *J. communis*, is found throughout the temperate portion of the northern hemisphere, and that these species parasitize from 10 to 51 species of aecial hosts in 3 to

12 genera, some of which are found in association with the telial host in all the northern continents. However, host associations alone do not seem sufficient to explain the peculiarly wide distribution of these species. The irregular zones of distribution shown on the maps would seem to indicate that temperature, humidity, and geographical factors are of much influence.

Gymnosporangium clavipes (Fig. 6) probably belongs in this category, but is limited in its geographical range to North America. While this is primarily an eastern North American species it has several stations in western North America and is not limited in its range by either alternate host group. Its alternate hosts, including *J. communis*, *J. virginiana*, and many of its more than 400 aecial-host species, occur in association throughout the greater portion of the northern hemisphere. It appears that this species has potentialities for increasing its geographical range. Since new stations are appearing in western North America from time to time, it would appear that it is actively doing so. This phenomenon has not been observed with any other species of the genus.

Discussion

Bisby (3) concludes from his analyses of geographical distribution of fungi that, in general, fungi have a wider distribution than flowering plants. The investigation on geographical distribution of the genus *Gymnosporangium* would seem to support conclusions quite the reverse of Dr. Bisby's general finding, as indicated by the three following observations. (i) Few species of *Gymnosporangium* occupy all potential territory covered by the coinciding ranges of their alternate hosts, which in each instance is of limited extent (Figs. 21, 22, 28, 29, and 30). (ii) Although the tricontinental species are of wide distribution, they fall far short of occupying all potential territory, They seem to be most abundant in Europe and of local occurrence in North America and Asia (Figs. 1, 2, and 3). (iii) Most species of this genus are of local distribution. They are not limited by the coinciding ranges of their alternate hosts and few species are limited geographically by one or the other of their hosts. With two well established exceptions only, *G. haraeanum* and *G. japonicum*, species are confined to a single continent.

Overholts (13, p. 631) is of the opinion that temperature and moisture must be among the predominant factors influencing the geographical distribution of certain polypores. There seems little doubt but that Overholts' evaluation of factors influencing the geographical distribution of polypores in North America is also largely applicable to the genus *Gymnosporangium*. But there seem to be additional limiting factors of much importance to this genus. All species of this genus are obligate parasites and all but two lack the repeating uredial stage; hence they can survive only within the area occupied by the coinciding ranges of their alternate hosts.

Temperature acting upon the parasite does not seem to be of paramount importance as a limiting factor. This is *indicated* by the finding that spores of many species will germinate over a greater range than that in which the

species live. Humidity acting upon the parasite also does not seem to be of paramount importance as a limiting factor. If humidity conditions are not favourable, even over the whole range of most species for one or a few years, the perennial nature of the parasite in the telial host will offset such an abnormal occurrence. A brief period of susceptibility during which aecial hosts can become infected has been shown to exist for certain species of *Gymnosporangium*, and one is assumed to exist for all aecial hosts of this genus of rusts. This factor is probably a potent one in regulating geographical range. If a species has a large number of aecial hosts with varying durations of the period of susceptibility and varying lateness for breaking bud, the time over which infection can occur is thereby lengthened and the potential geographical range extended. The reverse situation would also seem to be true.

It has been observed that the maturation of teliospores in the spring does not coincide with exactness with the production of new susceptible growth by aecial hosts. The writer's observations show that teliospores of *G. clavipes* may be germinable one to four weeks prior to the breaking of buds of aecial hosts under conditions in Massachusetts. In 1932, showers were so frequent, after teliospores of *G. clavipes* were germinable, that the majority of teliospores had germinated before buds of aecial hosts had broken; hence few were left to cause infection. So few, in fact, that *Aronia melanocarpa*, a susceptible shrub that produced foliage and flowers rather late in the spring, was not infected that year in the several areas visited, where plants were infected the year previous and in later years. The phenomenon of maturation and germination of teliospores prior to production of susceptible growth by aecial hosts has been observed with several species of *Gymnosporangium* in Massachusetts and New Mexico.

A species could not survive in any area where teliospores would regularly mature much later than buds break on aecial hosts, for the brevity of the period of susceptibility would render the new growth non-susceptible.

Geographical barriers are potent obstacles to the geographical ranges of most species of *Gymnosporangium*. These barriers, however, seem to have their effect by restricting the range of hosts, principally of the primary telial host. Primary telial hosts are endemic to each of the geographical areas discussed in the body of this paper, and certain species that are largely confined to one of these hosts are also restricted to its range. *Juniperus communis* is the only telial host to occur circumpolarly. It is interesting to observe that three rusts on this host are also circumpolar. However, *G. davisii*, which parasitizes *J. communis*, is of local distribution, while *G. clavipes*, which consistently parasitizes *J. communis* (and *J. virginiana*), is widely distributed in North America, though principally in the eastern region.

From the writer's observations to date it appears that no one factor is paramount in importance in governing the range of most species of *Gymnosporangium*. It appears vital that several phenomena, perhaps especially suitable temperature and humidity conditions and the existence of germinable

teliospores, must coincide with annual regularity during the period of susceptibility of aecial hosts in order that a species maintain itself within a geographical area where alternate hosts are associated.

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SEEDLING REACTIONS OF WHEAT VARIETIES TO STEM RUST AND LEAF RUST AND OF OAT VARIETIES TO STEM RUST AND CROWN RUST[1]

BY MARGARET NEWTON[2], T. JOHNSON[3], AND B. PETURSON[4]

Abstract

A study of the rust reactions of wheat varieties to 20 physiologic races of stem rust has shown that several varieties of the *vulgare* type, namely, McMurachy, Eureka, and several strains from Kenya, East Africa, are immune in the seedling stage at ordinary greenhouse temperatures (55° F. to 80° F. daily). This immunity largely disappears when the plants are kept at a constant high temperature (75° F. to 80° F.). Certain other varieties and hybrid strains were found rather highly resistant to eight physiologic races of leaf rust, but none of the *vulgare* varieties tested showed immunity or high resistance to both stem rust and leaf rust.

Tests to determine the resistance of oat varieties to the physiologic races of oat stem rust and crown rust prevalent in Canada showed that four oat varieties derived from the cross Hajira × Joanette were resistant to all the physiologic races of oat stem rust used in the test, and that certain strains derived from the cross Victoria × (Hajira × Banner Sel. 524) were resistant to all but one of these races. The last mentioned strains and the varieties Victoria and Trispernia proved resistant to the nine races of crown rust to which they were tested.

Introduction

The work reported in the present paper was undertaken mainly to assist the plant breeders of the Dominion Rust Research Laboratory and other Canadian plant geneticists in their plant breeding projects. There were two ways in which this could be done: first, by testing the rust resistance of varieties from various sources in the hope of discovering suitable breeding material; second, by determining the relative rust resistance of different lines of the hybrid populations developed by the plant breeders.

Although varieties are ultimately selected for rust resistance on the basis of their reaction to rust in the field, it is nevertheless important to supplement field work with studies conducted in the greenhouse where each variety can be studied in the seedling stage for its reaction to the various physiologic races of a rust. Reactions of varieties in the seedling stage to rust may be determined quickly and accurately, and because varieties found resistant

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to a physiologic race in the seedling stage are also resistant to it in the adult stage it is often possible, by means of such tests, to predict the field reaction of a variety with considerable accuracy.

This method of determining rust resistance appears to be fully satisfactory for oats but has its limitations in tests with wheat varieties because the seedling and adult plants of many wheat varieties do not react identically to stem rust, a fact noted by several earlier investigators including Melchers and Parker (12), Harrington and Aamodt (5), Hursh (8), Hayes, Stakman, and Aamodt (6), and Aamodt (1). A similar difference between the reactions of the seedling and adult plants of some wheat varieties to leaf rust was noted by Johnston and Melchers (9) who found that rust resistance increased as the plants advanced towards maturity. Such discrepancy between seedling and mature-plant tests is exemplified by the stem-rust reactions of many hybrid strains derived from crosses of common wheats with Hope and H 44. These strains, though completely or moderately susceptible in the seedling stage are highly resistant in later stages of growth. They possess what has been described by Goulden, Neatby and Welsh (4) as "mature-plant resistance".

Owing to the fact that the plant breeding program in connection with which this work was undertaken includes the development of wheat varieties resistant to wheat stem rust and leaf rust, and oat varieties resistant to oat stem rust and crown rust, it was found necessary to work with all these rusts. As it was not possible to include in these tests all of the physiologic races of these rusts occurring in Canada, the tests were necessarily confined to certain selected races. In the selection of these races two criteria were kept in mind: first, to include all of the races most prevalent in Canada in the last few years, and second, to include races of diverse pathogenic characteristics. The twenty selected races of wheat stem rust (*Puccinia graminis Tritici* Erikss. and Henn.) therefore included races such as 11, 17, 19, 21, 29, 32, 34, 36, 38, 39, and 56, which have been collected frequently in the field (16), and races such as 9, 15, and 120, which have seldom been collected but possess a wide pathogenic range. The tests with wheat stem rust may be regarded as a continuation of similar work reported in previous papers (17, 18). The present tests, however, include a number of varieties that have been produced since the time of the earlier work as well as several physiologic races of stem rust not known at that time, including race 56 which has in recent years become the predominant race in Canada and the United States.

Most of the varieties subjected to these tests were also tested for their reaction to eight physiologic races of leaf rust of wheat (*Puccinia triticina* Erikss.) including some of the races most prevalent in Canada, such as races 1, 9, 52, and 76. The fact that the same varieties were tested for their reaction to physiologic races of both stem rust and leaf rust makes it possible to select varieties on the basis of their resistance to both rusts.

The infection tests with oat varieties included all the known races of oat stem rust (*Puccinia graminis Avenae* Erikss. and Henn.) with the exception

of race 9 which has only been collected once in Canada, and race 11 which is a European race resembling race 10*a* rather closely. A number of the varieties tested have been previously studied for their seedling reactions to 10 physiologic races by Levine and Smith (10). The present tests embrace, however, many varieties not tested by them including several recently developed in Canada. Most of these varieties were also tested for their reaction to nine physiologic races of crown rust (*Puccinia coronata Avenae* Erikss. and Henn.). These races include all the ones commonly prevalent in Canada (16, 19) as well as race 45, which has been collected only twice in Canada but which is pathogenic to the variety Bond now widely used in breeding on account of its high resistance to most other crown rust races.

Methods

In all the varieties tested, the plants were grown in 4-in. pots, about ten seedlings per pot. The first seedling leaf of each plant was inoculated when 3 to 4 in. high. Following inoculation, the plants were kept in moist chambers for 24 hr., after which they were placed on benches in the greenhouse where they remained until notes were taken on their rust reactions. Two pots of seedlings were used for each inoculation so that the reactions of about twenty seedlings were obtained for each physiologic race employed. When the reaction of a variety to any race was in doubt, the whole test was repeated. The reactions were recorded in terms of the system originally devised by Stakman and Levine (20) and modifications of it were used for the other rusts (2, 7, 11, 13, 21, 22).

Owing to the fact that this method of recording rust reactions involves the use of symbols not familiar to all plant breeders, it was decided to translate these into terms of resistance and susceptibility so that the relative resistance of the varieties might be grasped more readily. This has been accomplished by means of the following scale in which a valuation in terms of rust resistance is given to the various symbols used for recording the infection types:

Symbol for infection type	Valuation in terms of rust resistance	Abbreviation
0 and 0;	= immunity	I
1, 2, 2+, x=, x−	= high resistance	R
2++, 3=, x	= moderate resistance	MR
3−, 3, x+, x++	= moderate susceptibility	MS
3+, 3++, 4	= complete susceptibility	S

It should be stated that the divisions between the different classes in the above scale are quite arbitrary. The justification of this procedure is that it facilitates a comparison of the relative resistance of two or more varieties. For the sake of convenience, the abbreviations shown above have been used in tabulating the rust reactions of the different wheat and oat varieties.

The Reaction of Wheat Varieties in the Seedling Stage to Physiologic Races of Stem Rust and of Leaf Rust

Stem Rust

Included in the tests of the reaction of wheat varieties to races of stem rust and leaf rust are some of the more promising of the rust-resistant wheats developed in recent years such as the stem-rust resistant varieties and strains developed in Canada from crosses of Marquis and Reward with Hope and H 44, and the Australian variety Eureka which was produced by Mr. S. L. Macindoe from the cross (Kenya Crossbred C 6040 × Florence) F_1 × Dundee and which has shown high stem-rust resistance in Australia. In addition, a number of varieties were tested that have no commercial value but that may possibly serve as useful breeding material, such as *Triticum Timopheevi* Zhuk. McMurachy*, and several strains developed in Kenya, East Africa.†

Among varieties known to possess some resistance to leaf rust may be mentioned a group of spring wheats supplied to the Dominion Rust Research Laboratory by Dr. R. M. Caldwell, Purdue University, Indiana. These wheats include Illinois No. 1 B. 8 and certain derivatives of the crosses Warden C.I. 4994-4 × Hybrid English W. 325, Chinese C.I. 6223 × Progress C.I. 6902, and Chinese C.I. 6223 × Emmer S.D. 293.

For purposes of comparison, tests were also made with a number of the older varieties such as Marquis, Huron, Preston, and Garnet, as well as the winter wheats Democrat, Hussar, Mediterranean, Malakoff, Fultz, and Kawvale.

The seedling reactions to stem rust and leaf rust are recorded in Tables I and II respectively. The varieties have been grouped according to the species to which they belong, but, within each species group, the varieties are arranged in approximately the order of their resistance—the most resistant varieties being placed first and the most susceptible last.

The tests with physiologic races of stem rust showed that, in the *vulgare* group, Eureka, the Kenya strains, and McMurachy are immune to all the races used, at least when tested in the seedling stage at ordinary greenhouse temperatures. A striking feature of the rust reaction of these varieties is the fact that this immunity disappears at high temperatures. The Kenya strains and McMurachy, when tested at ordinary greenhouse temperatures (varying from about 55° F. at night to 80° F. by day), developed, at most, minute flecks indicative of extreme resistance. When tested with the same race at a constant temperature of 75° to 80° F. they showed moderate or even complete susceptibility. Similar results were shown by plants approaching the boot stage of growth.

* *McMurachy was selected from a field of Garnet wheat, about 1930, by Mr. M. S. J. McMurachy of Strathclair, Manitoba. It was found to be resistant to stem rust and was brought to the attention of the plant breeders at the Dominion Rust Research Laboratory in 1935.*

† *The Kenya varieties were originally developed at Njoro, Kenya Colony, and were obtained by the Dominion Rust Research Laboratory in 1934. The identification numbers they bore at that time and the accession numbers given them at the Rust Research Laboratory were as follows: 122 D.I.T. (L)—R.L. 1373; 117 E.16.B.1—R.L. 1374; 117 B.5.B.2. (L)—R.L. 1375; 117 K.16.A (L)—R.L. 1376; 117 I.5.F. (L)—R.L. 1377.*

TABLE I

SEEDLING REACTIONS OF 78 WHEAT VARIETIES AND HYBRID STRAINS TO 20 PHYSIOLOGIC RACES OF *Puccinia graminis Tritici*

Variety	C.I. No.	R.L. No.	Physiologic race																			
			9	10	11	15	17	19	21	29	32	34	36	38	39	48	50	56	113	120	139	152
Triticum vulgare																						
Eureka		1534	–	I	I	I	I	I	I	I	I	I	I	I	I	I	–	I	I	I	I	I
Kenya		1373	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Kenya		1374	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Kenya		1375	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Kenya		1376	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Kenya		1377	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
McMurachy		1313	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Thatcher		1246	I	MS	MS	MR	I	I	I	I	I	MR	MR	MS	MS	I	I	MR	MR	I	I	R
Apex		1320	I	MR	MR	MS	I	I	I	I	MS	S	MS	MS	MS	I	I	MS	MR	I	I	MR
Canus		1321	–	R	S	S	I	I	I	I	S	S	S	R	R	R	–	S	S	I	I	R
Hope × Reward		1165	MS	R	MS	MS	MR	R	MS	MS	MS	MS	R	R	R	R	R	R	MR	MR	R	R
Hope		209	MS	R	MS	MS	MS	R	S	MS	MS	S	R	R	R	R	I	R	R	MR	R	R
H 44-24		229	MS	R	MR	MS	MS	R	MS	MS	MS	MS	R	R	R	MS	R	R	R	MR	R	R
Marquillo		132	MS	R	R	MS	MS	R	R	I	MS	MS	MS	R	MR	R	S	R	R	MR	MS	R
Renown Selection		716.6	MS	R	MS	S	MS	R	MS	MS	MS	S	R	R	R	R	R	R	R	R	R	R
Regent		975.1	MS	R	MS	MS	MS	R	MS	MS	MS	S	R	R	R	R	R	R	R	MS	R	R
Renown		716	MR	MR	MR	MS	MR	R	MS	MR	MS	MS	MR	R	R	MR	MS	MS	R	R	MR	R
Marquis × (H 44-24 × Marquis)		1333	MS	R	S	S	MS	R	S	S	MS	MS	R	R	R	R	R	R	MR	R	R	R
Reliance	7370	1813	I	S	S	S	I	I	I	I	S	S	S	S	MS	I	I	S	MR	I	R	MS
Coronation		729A	MS	R	S	MS	MS	R	S	S	MR	S	MR	R	R	R	R	MR	MR	R	R	R
H 44-24 × Reward		967	MR	MR	MR	MS	R	R	MR	MS	MS	MS	MR	MR	MR	MR	MS	MS	R	MR	MS	MR
H 44-24 × Marquis		1081	MS	MR	MS	MS	MS	R	MS	S	MS	MS	MS	MR	R	R	MR	R	MR	MR	MR	R
Marquis × (Pentad × Marquis)		1326	MS	R	S	S	MS	R	MS	S	MS	S	MS	R	R	R	R	S	MR	MR	R	R
H 44-24 × Reward		1097	MR	MR	MS	MS	MR	R	MS	MS	MR	S	S	MR	MR	MR	MS	MS	R	MR	MR	MR
H 44-24 × Marquis		704.1	MR	R	MS	MS	MR	R	MS	MS	MS	MS	MS	MR	MR	MR	MS	S	MR	MR	MS	MR
Webster	3780	365	MS	MR	MR	MS	MR	MS	MS	MS	MS	MS	MS	MR	MR	MS	MS	MR	R	MR	MR	MR
Haynes' Blue Stem		200	MS	R	S	S	MS	R	S	S	S	S	S	R	R	R	MR	S	R	R	MS	R
Marquis		572	S	R	S	S	S	R	S	S	S	S	S	R	R	R	R	S	MR	MR	R	R

TABLE I—*Continued*

SEEDLING REACTIONS OF 78 WHEAT VARIETIES AND HYBRID STRAINS TO 20 PHYSIOLOGIC RACES OF *Puccinia graminis Tritici*—*Continued*

Variety	C.I. No.	R.L. No.	Physiologic race																			
			9	10	11	15	17	19	21	29	32	34	36	38	39	48	50	56	113	120	139	152
***Triticum vulgare*—concluded**																						
Red Bobs		134	MS	R	S	S	S	R	S	S	S	S	S	R	R	R	MS	S	R	R	MS	R
Red Fife		65	MS	R	S	S	S	R	S	S	S	S	S	R	R	R	MS	S	R	R	MS	R
Ceres		127	S	MR	MS	MS	S	MS	S	S	MR	MS	S	MS	MR	I	MS	S	MR	R	MS	I
Early Triumph		94	–	R	S	S	S	R	S	S	S	S	S	R	R	R	–	S	R	MS	MS	R
Huron		20	MS	R	S	S	S	R	S	S	S	S	S	R	MS	R	MS	S	R	R	S	R
Preston		207	S	R	S	S	S	R	S	S	S	S	S	MR	R	R	MS	S	R	R	S	R
Kota	5878	571	S	R	S	S	S	MS	S	MS	S	S	S	MS	MS	R	R	S	MS	MR	R	I
Malakof	4898	1538	MR	MS	MR	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS
Kawvale		1451	–	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	–	MS	MS	MS	MS	MS
Loros	*3779*	*1891*	MS	MR	S	S	MS	I	S	S	MR	S	MS	MR	MR	S	S	S	MR	MS	MS	MS
Norka	4377	1888	MS	S	MR	R	S	MS	MR	R	S	R	R	S	S	MS	S	MS	S	S	S	MS
Warden × Hybrid		1803	–	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	–	MS	MS	MS	MS	MS
Glenwood		1411	S	S	S	S	S	S	S	S	R	S	S	MS	MS	I	MS	S	MS	R	MS	I
Hard Federation		1887	MS	R	S	S	S	MR	S	S	S	S	S	MR	MR	MR	S	S	MR	MR	S	R
Reward		79	S	MS	S	S	S	R	S	S	S	S	S	R	R	MR	MR	S	MR	S	MS	MR
Renfrew		135	S	MR	S	S	S	R	S	S	S	S	S	MR	MR	MR	–	S	S	MS	MS	R
Illinois No. 1.B. 8		1593	–	MS	S	S	S	MS	S	S	S	S	S	S	MS	MS	–	S	S	S	S	I
Carina	3756	1889	MS	MR	S	S	S	I	S	S	S	S	S	MR	MR	S	S	S	MR	S	S	S
Brevit	3778	1890	S	MR	S	S	S	MR	S	S	S	S	S	MR	MR	S	S	S	MR	S	S	S
Ridit		*1932*	–	MS	S	S	S	MS	S	S	S	S	S	MS	MS	MS	–	S	S	S	MS	MS
Hard Red Calcutta		1893	S	S	S	S	S	S	S	S	S	S	S	S	S	MS	S	S	S	S	S	S
Chinese × Emmer		1802	–	S	S	S	S	S	S	S	S	S	S	S	S	S	–	S	S	S	S	S
Chinese × Progress		1804	–	S	S	S	S	S	S	S	S	S	S	S	S	S	–	S	S	S	S	S
Chinese × Progress		*1805*	–	S	S	S	S	S	S	S	S	S	S	S	S	S	–	S	S	S	S	S
Democrat	3384	1535	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Fultz		1539	–	S	S	S	S	S	S	S	S	S	S	S	S	S	–	S	S	S	S	S
Fultz Selection		1540	–	S	S	S	S	S	S	S	S	S	S	S	S	S	–	S	S	S	S	S
Garnet		15	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Hussar	*4843*	*1892*	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Mediterranean	3332	1537	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Prelude		25	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

TABLE I—*Concluded*

SEEDLING REACTIONS OF 78 WHEAT VARIETIES AND HYBRID STRAINS TO 20 PHYSIOLOGIC RACES OF *Puccinia graminis Tritici*—*Concluded*

Variety	C.I. No.	R.L. No.	Physiologic race																			
			9	10	11	15	17	19	21	29	32	34	36	38	39	48	50	56	113	120	139	152
Triticum durum																						
Iumillo		7	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Iumillo × Mindum		1183	–	I	I	I	I	I	I	I	I	I	I	I	I	R	–	I	R	I	I	R
Belaturka		1412	–	R	R	R	R	R	R	R	MR	R	R	R	R	MR	–	R	R	R	MR	R
Pentad		203	MS	MS	R	R	MS	MS	MS	R	I	MR	R	R	MS	I	R	R	R	MR	I	I
Mindum	5296	568	S	S	S	S	S	S	S	MR	MR	S	R	MR	S	R	I	R	R	S	R	I
Spelmar	6236	569	S	S	S	S	S	S	S	MR	MR	S	R	MR	S	R	I	R	R	S	R	I
Tibet		1396	–	MS	MS	MS	MR	R	R	MR	S	MS	MR	MS	MR	MR	–	MS	R	R	S	MS
Akrona		1252	–	S	S	S	S	S	S	MR	MR	S	R	MR	S	MR	–	R	R	MR	R	R
Arnautka	4064	570	S	S	S	S	S	S	S	MS	MS	S	R	MS	S	MS	R	R	MS	S	R	R
Kubanka	2094	565	S	S	S	S	S	S	S	MR	R	S	MR	MS	S	MS	R	S	R	S	MR	R
Acme	5284	566	S	S	S	S	S	S	S	MS	MS	S	S	MS	S	S	MS	S	S	S	S	S
Pelissier		145	S	S	S	S	S	S	S	S	S	S	S	S	S	S	–	S	S	S	S	S
Triticum compactum																						
Jenkin	5177	1814	S	S	MS	S	MS	S	MS	S	MS	S	MS	S	S	S	MS	MS	S	S	MS	S
Little Club	4066	223	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Triticum dicoccum																						
Khapli	4013	563	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	I	R
Vernal	3686	567	S	R	R	S	R	I	I	R	R	I	I	R	R	R	I	R	I	MS	R	I
Black Persian		388	R	MR	R	R	R	R	R	R	R	R	R	R	MR	R	R	MR	MR	R	MR	R
Triticum monococcum																						
Einkorn	2433	227	S	S	MS	S	MS	MS	R	MS	MS	R	S	S	S	S	I	R	S	S	R	S
																					R	S
Triticum Timopheevi		1308	I	I	I	R	I	MR	R	I	I	I	R	R	I	R	I	MR	I	I	I	I

TABLE II

SEEDLING REACTIONS OF 75 WHEAT VARIETIES AND HYBRID STRAINS TO 8 PHYSIOLOGIC RACES OF *Puccinia triticina*

Varieties	C.I. No.	R.L. No.	Physiologic race							
			1	9	11	27	28	29	52	76
Triticum vulgare										
Warden × Hybrid English W 325		1803	R	I	R	R	R	I	R	R
Carina	3756	1889	I	R	R	R	R	MR	I	MR
Illinois No. 1.B 8	11628	1593	R	R	R	R	R	R	R	R
Kawvale	8180	1451	I	I	R	I	S	I	I	S
Fultz		1539	I	I	R	I	S	I	I	S
Chinese × Progress		1805	R	R	R	MR	R	MR	R	R
Fultz Selection		1540	I	I	R	I	S	R	I	S
Chinese × Progress		1804	R	R	R	MR	MR	MR	R	R
Chinese × Emmer S.D. 293		1802	R	R	MR	MR	MR	R	R	MS
Brevit	3778	1890	I	R	S	R	R	MR	R	S
Webster	3780	365	I	S	R	S	R	MR	I	MR
Hussar	4843	1892	R	R	R	MR	MS	MS	MS	R
Marquis × (H 44-24 × Marquis)		1333	MR	MR	MR	MR	R	MR	MR	MR
Hope		209	MR	MR	MR	MR	R	MR	MR	MR
Democrat	3384	1535	I	R	R	R	S	R	S	S
Hope × Reward		1165	MR	MR	MR	MR	MR	MR	MR	MR
H 44-24		229	MR	MR	MR	MR	MR	MR	MR	MR
Renown Selection		716.6	MR	MR	MR	MS	R	MR	MR	MR
Mediterranean	3332	1537	R	R	R	R	S	R	S	S
Coronation		729A	MR	MS	MR	MR	MR	MR	MR	MR
H 44-24 × Marquis		704.1	MR	MR	MR	MR	MR	MR	MR	MS
Renown		716	MR	R	MS	MR	MS	MR	MR	MR
Hard Federation		1887	MS	MR	S	MR	MR	R	R	MS
Malakof	4898	1538	I	S	I	S	S	S	S	I
Norka	4377	1888	I	S	I	S	S	S	S	I
Marquis × (Pentad × Marquis)		1326	MS	MR	MS	MR	MS	MR	MS	MR
Loros	3779	1891	I	S	S	MS	MS	S	R	S
H 44-24 × Marquis		1081	MS	MS	MR	MS	MS	MS	MS	MS
H 44 × Reward		975	MS	MS	MS	MS	MS	MS	MS	MS
H 44-24 × Reward		967	MS	S	MS	S	S	S	MS	MS
Reliance	7370	1813	S	S	MS	S	S	S	MS	MS
Haynes' Blue Stem		200	S	S	S	S	S	MS	MS	S
Glenwood		1411	S	S	MS	S	S	S	S	MS
Regent		975.1	S	S	S	MS	S	MS	S	S
Kenya		1376	S	S	S	S	MS	S	MS	S
Kenya		1375	S	S	S	S	MS	S	S	S
Preston		207	S	S	S	S	S	S	MS	S
Renfrew		135	S	S	S	S	S	S	MS	S
Apex		1320	S	S	S	S	S	S	S	S
Canus		1321	S	S	S	S	S	S	S	S
Ceres		127	S	S	S	S	S	S	S	S
Early Triumph		94	S	S	S	S	S	S	S	S
Garnet		15	S	S	S	S	S	S	S	S
Huron		20	S	S	S	S	S	S	S	S
Kenya		1373	S	S	S	S	S	S	S	S
Kenya		1374	S	S	S	S	S	S	S	S
Kenya		1377	S	S	S	S	S	S	S	S
Kota	5878	571	S	S	S	S	S	S	S	S
Marquillo		132	S	S	S	S	S	S	S	S
Marquis		572	S	S	S	S	S	S	S	S

TABLE II—*Concluded*

SEEDLING REACTIONS OF 75 WHEAT VARIETIES AND HYBRID STRAINS TO 8 PHYSIOLOGIC RACES OF *Puccinia triticina*—*Concluded*

Varieties	C.I. No.	R.L. No.	Physiologic race							
			1	9	11	27	28	29	52	76
Triticum vulgare—continued										
McMurachy		1313	S	S	S	S	S	S	S	S
Prelude		25	S	S	S	S	S	S	S	S
Red Bobs		134	S	S	S	S	S	S	S	S
Red Fife		65	S	S	S	S	S	S	S	S
Reward		79	S	S	S	S	S	S	S	S
H 44-24 × Reward		1097	S	S	S	S	S	S	S	S
Thatcher		1246	S	S	S	S	S	S	S	S
Triticum compactum										
Little Club	4066	223	S	S	S	S	S	S	S	S
Triticum durum										
Arnautka	4064	570	R	R	MR	R	R	R	R	R
Iumillo		7	R	R	MR	R	MS	R	R	R
Mindum	5296	568	MS	R	MR	R	R	R	R	R
Akrona		1252	MR	R	MS	R	MR	R	R	R
Iumillo × Mindum		1183	MS	R	S	R	R	MR	R	MR
Acme	5284	566	S	R	MR	R	MS	R	MS	MS
Spelmar	6236	569	S	R	S	R	MS	R	MR	MS
Pelissier		145	MR	MR	MS	R	S	MS	MR	MS
Pentad		203	MS	MR	MS	MR	MS	MR	MR	MS
Tibet		1396	MS	MR	S	MR	S	R	MS	MS
Belaturka		1412	MS	MR	S	MR	S	MR	MR	S
Kubanka	2094	565	S	MS	S	MR	S	R	MS	S
Triticum dicoccum										
Khapli	4013	563	MS	MR	MS	R	MR	MS	MS	MS
Vernal	3686	567	MS	MR	S	MS	MS	MS	MS	MS
Black Persian		388	S	S	R	S	MS	S	S	S
Triticum monococcum										
Einkorn	2433	227	R	I	R	R	R	R	R	R
Triticum Timopheevi		1308	I	I	I	I	I	I	I	I

Thatcher, Apex, and many of the varieties derived from crosses of common wheats with Hope and H 44, show a considerable amount of resistance to stem rust. It should be pointed out here that the seedling tests are not a fair indication of the rust reaction of Hope and H 44 derivatives, as these, in later stages of growth, develop the so-called mature-plant resistance. The field resistance of these varieties is, therefore, greater than that indicated by the seedling tests. This resistance cannot, however, be regarded as completely stable inasmuch as recent tests with Hope wheat have shown that adult plants kept at a constant high temperature (75° to 80° F.) are rendered moderately susceptible.

Among the non-*vulgare* varieties, only Iumillo and Iumillo × Mindum showed immunity. It should be noted, however, that when Iumillo was tested at constant high temperatures (75° to 80° F.) immunity was occasionally altered to moderate resistance (an "*x*" reaction). Several other varieties such as Pentad, Belaturka, Khapli, Black Persian, and *T. Timopheevi* were highly resistant at ordinary greenhouse temperatures.

Leaf Rust

In the tests with leaf rust of wheat, considerable resistance was shown by Illinois No. 1 B. 8 and certain derivatives of the crosses Warden × Hybrid English, Chinese × Progress, and Chinese × Emmer. These results were supported by evidence secured from adult plants of the same varieties subjected to a rather severe artificially induced epidemic in the field during the summer of 1939. They were found to have considerably more resistance to leaf rust than the varieties Fultz and Kawvale, which are generally regarded as at least moderately resistant (3, 9, 24). Besides, in the greenhouse tests, Fultz and Kawvale proved completely susceptible to two of the physiologic races. A moderate resistance was shown by several other varieties, such as Carina, Brevit, Webster, Hope, H 44, and a number of derivatives of crosses with the two last-mentioned varieties. Varieties descended from Hope and H 44, however, are, in the later stages of growth, somewhat more resistant to leaf rust, so that the seedling reaction is not an exact indication of the field reaction. The variety McMurachy and the Kenya strains which proved immune to stem rust are susceptible to leaf rust. Of the varieties tested, the ones showing the greatest resistance to both stem rust and leaf rust appear to be *Triticum Timopheevi* and the *durum* variety Iumillo.

The Reaction of Oat Varieties in the Seedling Stage to Physiologic Races of Oat Stem Rust and of Crown Rust

Stem Rust

The oat varieties studied for their reaction to physiologic races of oat stem rust included many new varieties developed in recent years in Canada and the United States. The rust reactions of these varieties are recorded in Table III. On the basis of these reactions, the varieties are divided into the following six groups:

(1) A group consisting of several strains derived from the cross Hajira × Joanette. These strains are resistant or moderately resistant to all the 11 physiologic races used in these tests.

(2) A group comprising strains derived from the crosses Hajira × Banner and Victoria × (Hajira × Banner Sel. 524), which show resistance to all races except race 6. These together with those of the first group were developed by Mr. J. N. Welsh of the Dominion Rust Research Laboratory.

(3) Varieties reacting like Richland, and including Monarch Selection, Hajira, Rusota, Iogold, Vanguard, Green Russian N.D. 22005, and several strains derived from the cross Victoria × (Hajira × Banner Sel. 524). These are resistant to races 1, 2, 3, 5, 7, and 12.

TABLE III

SEEDLING REACTIONS OF 78 OAT VARIETIES AND HYBRID STRAINS TO 11 RACES OF *Puccinia graminis Avenae*

Varieties	C.I. No.	R.L. No.	Physiologic race 1	2	3	4	5	6	7	8	10	10a	12	13
Avena sativa														
Hajira × Joanette		811	R	R	I	R	R	R	R	R	R	R	R	R
Hajira × Joanette		800	R	R	I	R	R	MR	R	R	R	R	R	R
Hajira × Joanette		824	R	R	I	R	R	MR	R	R	R	R	R	R
Hajira × Joanette		818	R	R	I	MR	R	MR	R	R	R	R	R	R
Victoria × S-524* Sel. 1225			R	R	R	R	R	MS	R	R	R	R	R	R
Victoria × S-524 Sel. 1228			R	R	R	R	R	MS	R	R	R	R	R	R
Victoria × S-524 Sel. 1273			R	R	R	R	R	MS	R	R	R	R	R	R
Victoria × S-524 Sel. 1282			R	R	R	R	R	MS	R	R	R	R	R	R
Hajira & Banner Sel. 524			R	R	R	R	R	S	R	R	R	R	R	R
Joanette Strain	2660	561	R	S	R	R	MR	S	S	S	MS	R	MR	MR
Sevnothree	3251	562	R	S	R	R	MR	S	S	S	MS	R	MR	MR
Hajira	1001	559	R	R	R	S	R	S	R	S	S	S	R	S
Iogold	2329	387	R	R	R	S	R	S	R	S	S	S	R	S
Green Russian, N.D. 22005		1297	R	R	R	S	R	S	R	S	S	S	R	S
Monarch Sel.	1879	363	R	R	R	S	R	S	R	S	S	S	R	S
Richland	787	172	R	R	R	S	R	S	R	S	S	S	R	S
Rusota	2343	371	R	R	R	S	R	S	R	S	S	S	R	S
Vanguard		7	R	R	R	S	R	S	R	S	S	S	R	S
Victoria × S-524 Sel. 909			R	R	R	S	R	S	R	S	S	S	R	S
Victoria × S-524 Sel. 921			R	R	R	S	R	S	R	S	S	S	R	S
Victoria × S-524 Sel. 922			R	R	R	S	R	S	R	S	S	S	R	S
Anthony	2143	1075	MR	MR	S	S	MR	S	S	MR	MR	MR	S	S
Green Russian	2890	1093	MR	MR	S	S	MR	S	S	MR	MR	MR	S	S
Minnesota 439		243	MR	MR	S	S	MR	S	S	MR	MR	MR	S	S
Minrus	2144	1078	MR	MR	S	S	MR	S	S	MR	MR	MR	S	S
Abundance		844	S	S	S	S	S	S	S	S	S	S	S	S
Alaska		165	S	S	S	S	S	S	S	S	S	S	S	
Awnless Rust Proof	1776	773	S	S	S	S	S	S	S	S	S	S	S	S
Banner		1301	S	S	S	S	S	S	S	S	S	S	S	S

* *S-524 = Selection from (Hajira × Banner).*

TABLE III—*Continued*

SEEDLING REACTIONS OF 78 OAT VARIETIES AND HYBRID STRAINS TO 11 RACES OF *Puccinia graminis Avenae*—*Continued*

Varieties	C.I. No.	R.L. No.	Physiologic race											
			1	2	3	4	5	6	7	8	10	10a	12	13
Avena sativa—concluded														
Banner		179	S	S	S	S	S	S	S	S	S	S	S	S
Black Mesdag	1877	784	S	S	S	S	S	S	S	S	S	S	S	S
Cartier		633	S	S	S	S	S	S	S	S	S	S	S	S
Eagle		1131	S	S	S	S	S	S	S	S	S	S	S	S
Erban		1307	S	S	S	S	S	S	S	S	S	S	S	S
Gopher	2027	158	S	S	S	S	S	S	S	S	S	S	S	S
Gold Rain		827	S	S	S	S	S	S	S	S	S	S	S	S
Lanark		1306	S	S	S	S	S	S	S	S	S	S	S	S
Legacy		193	S	S	S	S	S	S	S	S	S	S	S	S
Mabel		1314	S	S	S	S	S	S	S	S	S	S	S	S
Markton	2053	353	S	S	S	S	S	S	S	S	S	S	S	S
Minnesota 295	1295	839	S	S	S	S	S	S	S	S	S	S	S	S
Minota	1285	911	S	S	S	S	S	S	S	S	S	S	S	S
O.A.C. 72		168	S	S	S	S	S	S	S	S	S	S	S	S
O.A.C. 144		171	S	S	S	S	S	S	S	S	S	S	S	S
Trispernia		3	S	S	S	S	S	S	S	S	S	S	S	S
Victory	1145	159	S	S	S	S	S	S	S	S	S	S	S	S
60-Day	1887	902	S	S	S	S	S	S	S	S	S	S	S	S
Avena diffusa														
		1023	S	S	S	S	S	S	S	S	S	S	S	S
		1027	S	S	S	S	S	S	S	S	S	S	S	S
		1028	S	S	S	S	S	S	S	S	S	S	S	S
		1034	S	S	S	S	S	S	S	S	S	S	S	S
Avena sativa orientalis														
Green Mountain	1892	929	MR	MR	S	S	MR	S	S	MR	MR	MR	S	S
White Tartar	551	177	MR	MR	S	S	MR	S	S	MR	MR	MR	S	S
Avena barbata														
C.A.N. 221		1013	S	S	S	S	S	S	S	S	S	S	S	S
C.A.N. 220		1014	S	S	S	S	S	S	S	S	S	S	S	S

TABLE III—*Concluded*

SEEDLING REACTIONS OF 78 OAT VARIETIES AND HYBRID STRAINS TO 11 RACES OF *Puccinia graminis Avenae*—*Concluded*

Varieties	C.I. No.	R.L. No.	Physiologic race 1	2	3	4	5	6	7	8	10	10a	12	13
Avena byzantina														
Belar	2760	1310	S	S	S	S	S	S	S	S	S	S	S	S
Bond	2733	1130	S	S	S	S	S	S	S	S	S	S	S	S
Early Ripe		161	S	S	S	S	S	S	S	S	S	S	S	S
Early Ripe		634	S	S	S	S	S	S	S	S	S	S	S	S
Early Ripe 213 M.C.		1303	S	S	S	S	S	S	S	S	S	S	S	S
Kanota	839	805	S	S	S	S	S	S	S	S	S	S	S	S
Red Rust Proof	1815	1311	S	S	S	S	S	S	S	S	S	S	S	S
Ruakura	2025	345	S	S	S	S	S	S	S	S	S	S	S	S
Sunrise	982	1318	S	S	S	S	S	S	S	S	S	S	S	S
Victoria	2401	1006	S	S	S	S	S	S	S	S	S	S	S	S
Sterisel	2891	1227	S	S	S	S	S	S	S	S	S	S	S	S
Avena fatua		18	S	S	S	S	S	S	S	S	S	S	S	S
Avena nuda														
Laurel		167	S	S	S	S	S	S	S	S	S	S	S	S
Liberty	845	760	S	S	S	S	S	S	S	S	S	S	S	S
Avena brevis														
	1783		S	S	S	S	S	S	S	S	S	S	S	S
		1018	S	S	S	S	S	S	S	S	S	S	S	S
		1019	S	S	S	S	S	S	S	S	S	S	S	S
		1020	S	S	S	S	S	S	S	S	S	S	S	S
		1022	S	S	S	S	S	S	S	S	S	S	S	S
Avena strigosa														
	1782	1319	S	S	S	S	S	S	S	S	S	S	S	S
		1041	S	S	S	S	S	S	S	S	S	S	S	S
		1045	S	S	S	S	S	S	S	S	S	S	S	S
Glabrota	2630	1312	S	S	S	S	S	S	S	S	S	S	S	S

(4) Varieties reacting like White Tartar, and including Anthony, Green Mountain, Minrus, Minnesota 439, Green Russian C.I. 2890, and Victory × Black Mesdag. These are moderately resistant to races 1, 2, 5, 8, 10, and 10*a*.

(5) Joanette Strain and Sevnothree which are resistant to races 1, 3, 4, and 10*a* and moderately resistant to races 5, 12, and 13.

(6) Varieties susceptible to all physiologic races. These include the majority of the varieties tested.

Crown Rust

The tests with physiologic races of crown rust are summarized in Table IV Of the varieties tested, the only ones that showed resistance to all nine of the physiologic races employed are Victoria, Trispernia (a recent introduction from Roumania by the Cereal Division, Department of Agriculture, Ottawa), and a number of pure lines derived from the cross Victoria × (Hajira × Banner Sel. 524). Another highly resistant oat is the variety Bond (*A. byzantina*), which showed immunity to all races except the rarely occurring race 45 to which it proved highly susceptible. The resistance of Victoria and Bond has already been reported by Murphy, who found Victoria resistant to 37 physiologic races (14) and Bond immune to six physiologic races including four races not used in the present tests (13). Considerable resistance was also shown by the varieties Ruakura and Glabrota (*A. strigosa*), each of which was found susceptible to only two of the races used in the tests. All other varieties proved susceptible to five or more of the nine races to which they were tested.

A comparison of Tables III and IV will show that the crown-rust resistant varieties Trispernia, Victoria, Bond, Ruakura, and Glabrota are highly susceptible to stem rust. A combination of high resistance to stem rust and to crown rust is, however, possessed by certain of the Victoria × S-524 hybrids. Resistance to these two rusts appears also to have been built up by Stanton *et al.* (23) through crosses of Victoria × Richland and by Murphy *et al.* (15) through crosses of Bond with Iogold and other varieties.

Discussion

The study on the rust reaction of seedlings reported in the present paper shows that there is no lack of available rust-resistant breeding material in either wheat or oats. In *Triticum vulgare* and *Avena sativa* alike, there exist varieties that are highly resistant to all of the physiologic races of their respective stem rusts now common in North America. The tests with leaf rust of wheat were less extensive than those with stem rust inasmuch as only eight physiologic races were used; but as far as can be judged from these tests there exist several varieties of common wheat with a high degree of resistance to this rust.

In the tests with stem rust of wheat, the Australian variety Eureka, a group of strains from Kenya, and the Canadian variety McMurachy show great promise as breeding material owing to their high resistance (immunity

TABLE IV

SEEDLING REACTIONS OF 74 OAT VARIETIES AND HYBRID STRAINS TO 9 RACES OF *Puccinia coronata Avenae*

Variety	C.I. No.	R.L. No.	Physiologic race								
			1	2	3	4	5	6	24	38	45
Avena sativa											
Victoria × S-524* Sel. 1228			R	I	I	R	R	I	I	I	R
Victoria × S-524 Sel. 1273			R	I	I	R	R	I	I	I	R
Victoria × S-524 Sel. 914			R	I	R	R	R	I	I	I	R
Victoria × S-524 Sel. 909			R	R	I	R	R	I	I	I	R
Victoria × S-524 Sel. 907			R	R	R	R	R	I	I	I	R
Victoria × S-524 Sel. 921			R	R	R	R	R	I	I	I	R
Victoria × S-524 Sel. 922			R	R	R	R	R	I	I	I	R
Victoria × S-524 Sel. 1225			R	I	I	R	R	I	R	R	R
Trispernia		3	R	I	R	R	R	R	R	R	R
Joanette	2660	561	S	R	MR	S	S	R	I	I	S
Hajira × Joanette		800	S	R	MR	S	S	R	R	R	S
Hajira × Joanette		818	S	R	MR	S	S	R	R	R	S
Hajira × Joanette		824	S	R	MR	S	S	R	R	R	S
Anthony	2143	1075	S	I	S	I	I	S	S	I	S
Green Russian	2890	1093	S	I	S	I	I	S	S	I	S
Minrus	2144	1078	S	R	S	R	I	S	S	R	S
Mabel		1304	S	R	R	S	S	S	I	R	S
Lanark		1306	S	R	R	S	S	S	I	R	S
Victoria × S-524 Sel. 1282			S	S	S	MS	MS	R	R	R	S
Minota	1285	911	S	S	S	S	S	MS	S	S	S
Abundance		844	S	S	S	S	S	S	S	S	S
Alaska		165	S	S	S	S	S	S	S	S	S
Awnless Rustproof	1776	773	S	S	S	S	S	S	S	S	S
Banner		1301	S	S	S	S	S	S	S	S	S
Black Mesdag	1877	784	S	S	S	S	S	S	S	S	S
Cartier		633	S	S	S	S	S	S	S	S	S
Eagle		1131	S	S	S	S	S	S	S	S	S
Erban		1703	S	S	S	S	S	S	S	S	S
Gold Rain		827	S	S	S	S	S	S	S	S	S
Gopher	2027	158	S	S	S	S	S	S	S	S	S
Hajira	1001	559	S	S	S	S	S	S	S	S	S
Hawkeye	2264	1313	S	S	S	S	S	S	S	S	S
Iogold	2329	387	S	S	S	S	S	S	S	S	S
Legacy		193	S	S	S	S	S	S	S	S	S
Markton	2053	353	S	S	S	S	S	S	S	S	S
Minnesota 295	1295	839	S	S	S	S	S	S	S	S	S
Monarch		560	S	S	S	S	S	S	S	S	S
O.A.C. 72		168	S	S	S	S	S	S	S	S	S
O.A.C. 144	-	171	S	S	S	S	S	S	S	S	S
Richland	787	172	S	S	S	S	S	S	S	S	S
Vanguard		7	S	S	S	S	S	S	S	S	S
Victory	1145	159	S	S	S	S	S	S	S	S	S
Sixty-Day	1887	902	S	S	S	S	S	S	S	S	S
Avena diffusa											
		1023	S	S	S	S	S	S	S	S	S
		1027	S	S	S	S	S	S	S	S	S
		1028	S	S	S	S	S	S	S	S	S
		1034	S	S	S	S	S	S	S	S	S
Avena sativa Orientalis											
Green Mountain	1892	929	S	I	S	I	I	S	S	I	S
White Tartar	551	177	S	I	S	I	I	S	S	I	S

* *S-524 = Selection from (Hajira × Banner).*

TABLE IV—*Concluded*

SEEDLING REACTIONS OF 74 OAT VARIETIES AND HYBRID STRAINS TO 9 RACES OF *Puccinia coronata Avenae*—*Concluded*

Variety	C.I. No.	R.L. No.	Physiologic race								
			1	*2*	*3*	*4*	*5*	*6*	*24*	*38*	*45*
Avena barbata											
C.D. 997			S	S	S	S	S	S	S	S	S
A. barbata C.A.N. 221		1013	S	S	S	S	S	S	S	S	S
A. barbata C.A.N. 220		1014	S	S	S	S	S	S	S	S	S
Avena byzantina											
Victoria	2401	1006	R	I	I	R	R	I	I	I	R
Bond	2733	1130	I	I	I	I	I	I	I	I	S
Ruakura	2025	345	S	I	I	S	R	I	I	I	MR
Red Rustproof	1815	1311	S	I	I	S	S	S	I	I	S
Sterisel	2891	1227	S	I	I	S	S	S	I	I	S
Sunrise	982	1318	S	I	I	S	S	S	I	I	S
Early Ripe 213 M.C.		1303	S	I	R	S	S	S	R	R	S
Belar	2760	1310	S	R	R	S	S	S	R	R	S
Kanota	839	805	S	S	S	S	S	MS	S	S	S
Early Ripe		634	S	S	S	S	S	S	S	S	S
Early Ripe		161	S	S	S	S	S	S	S	S	S
Avena fatua		18	S	S	S	S	S	S	S	S	S
Avena nuda											
Laurel		167	S	S	S	S	S	S	S	S	S
Liberty	845	760	S	S	S	S	S	S	S	S	S
Avena brevis											
	1783		S	S	S	S	S	S	S	S	S
		1018	S	S	S	S	S	S	S	S	S
		1019	S	S	S	S	S	S	S	S	S
		1020	S	S	S	S	S	S	S	S	S
		1022	S	S	S	S	S	S	S	S	S
A. strigosa											
Glabrota	2630	1312	I	I	I	I	I	I	S	S	I
		1041	S	S	S	S	S	S	S	S	S
		1045	S	S	S	S	S	S	S	S	S
	1782	1319	S	S	S	S	S	S	S	S	S

t ordinary temperatures) to all the 20 physiologic races used in the tests. 'he immunity possessed by non-*vulgare* varieties, such as Iumillo, is of less ractical importance to plant breeders on account of the difficulty of crossing hem with *vulgare* wheats and the danger of introducing undesirable non-*ulgare* characters into the hybrid progeny. Quite apart from the immunity r near-immunity possessed by the above mentioned varieties, there is available to plant breeders the so-called mature-plant resistance possessed by arieties derived from crosses with Hope and H 44. The resistance to stem

rust of such varieties is much greater than the seedling reactions indicate owing to the tendency on the part of these varieties to become more resistant in the more advanced stages of growth.

As pointed out elsewhere in this paper, all the above-mentioned varieties failed to show their characteristic rust reactions at constant high temperatures. The immunity of McMurachy, Eureka, and the Kenya strains was altered to moderate or even complete susceptibility; the mature-plant resistance of Hope, to moderate resistance or moderate susceptibility; and the immunity of Iumillo was occasionally altered to moderate resistance. In view of these results it seems possible that the diminution of rust resistance by high temperatures is a general phenomenon applicable to many varieties. The breakdown of the immunity or resistance of these varieties at high temperatures is probably not of great significance in respect to their field resistance, except perhaps in regions where temperatures are excessively high for considerable periods.

Unfortunately, the *vulgare* varieties most highly resistant to stem rust are susceptible to leaf rust, but, as there exist in the *vulgare* group varieties with high resistance to leaf rust, there should be no great difficulty in combining resistance to leaf rust and stem rust. Hybrid progeny possessing apparently resistance to these two rusts has, in fact, been obtained recently at the Dominion Rust Research Laboratory from crosses of the stem-rust resistant varieties Kenya R.L. 1373 and McMurachy with derivatives of H 44 that are resistant to leaf rust.

The tests conducted on the reaction of oat varieties to physiologic races of oat stem rust and of crown rust should furnish a valuable guide to the field reaction of the varieties tested owing to the fact that, in oats, the reactions of the seedling and the mature plant resemble each other closely. As these tests comprised all but two of the known races of oat stem rust and most of the prevailing races of crown rust, they may be regarded as quite comprehensive. From the tests with stem rust, it appears that oat varieties show less variability in their reactions to stem rust than do wheat varieties. In fact, as already pointed out, the oat varieties tested may be divided into six groups according to their stem-rust reactions. In two of these groups are found varieties with high stem-rust resistance. Derivatives of the cross Hajira × Joanette are resistant to all the physiologic races used in these tests, and certain strains descended from the cross Victoria × (Hajira × Banner Sel. 524) are resistant to all but one of these races. The latter strains possess the further advantage of being highly resistant to crown rust.

In the tests with crown rust, the varieties Victoria and Trispernia, and the above mentioned hybrid lines descended from the cross Victoria × (Hajira × Banner Sel. 524), showed resistance to the nine races used in the tests, while the variety Bond proved immune to eight of these races.

Of all the oat varieties dealt with in the present paper, the only ones combining a high resistance to crown rust and stem rust are the Victoria × (Hajira × Banner Sel. 524) hybrid lines.

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EFFECT OF PHYTOHORMONE DUST SEED TREATMENT ON GROWTH AND YIELD OF BARLEY UNDER GREENHOUSE CONDITIONS[1]

By J. W. Hopkins[2]

Abstract

Under controlled conditions of air temperature and soil moisture, the growth and yield of barley from seed dusted with 2.5 and 5 p.p.m. of indolylacetic acid in talc was compared with that from control seed dusted with talc only. Neither concentration affected germination. Both accelerated the incidence of tillering by one or two days, but did not increase the final number of shoots produced. With sub-optimal soil moisture both phytohormone concentrations increased the height of the plants by about 3% and the dry weight of straw by about 10%, but did not increase the yield of grain. No demonstrable effect upon the yield of either straw or grain was observed in a parallel series of plants receiving a more plentiful supply of moisture.

Introduction

Although many details still remain to be investigated, the physiological activity of minute concentrations of the so-called phytohormone chemicals in promoting the rooting of plant cuttings of certain species is now definitely established. The circumstances in which the application of phytohormones to seeds prior to planting may be expected to stimulate the subsequent growth of field and garden crops are, however, as yet uncertain. Stier and duBuy (3), for example, report statistically significant acceleration of flowering and increase in early and total yield of tomatoes following treatment of the seed with auxin-talc dust mixtures and subsequent dipping of the roots into solutions of either indolylbutyric or naphthylacetic acid at the time of field transplanting. On the other hand, Templeman (4) noted no significant increase in the dry-matter production of barley or mustard grown with restricted or ample nitrogen supply following the application of phytohormone substances, either by spraying on the foliage or by watering. Soaking mustard seed for 24 hr. in solutions of phytohormone before planting likewise failed to result in any stimulation of subsequent growth.

Greenhouse experiments in these laboratories by Grace (1) suggested some increase in the germination and early growth of wheat from the treatment of the seed with carrier dusts containing small quantities of phytohormones. However, the subsequent results of a fairly extensive series of co-operative field trials indicated that although indolylacetic and naphthylacetic acids (the two substances tested) are potentially capable of physiological activity when applied in this way (2), yet in practice these potentialities are manifested only under conditions that are of rather restricted occurrence. Why this should be so is not yet clear, but it seems reasonable to suppose that the

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operation of environmental factors is at least partly responsible. If, for example, the primary effect of phytohormones is to stimulate root formation, it is conceivable that their application might prove to be most advantageous to a crop growing under moderately sub-optimal moisture conditions; for if the supply is abundant, the intensive exploitation of soil moisture by the root system will not be a factor limiting yield, and in the event of extreme drought, crop failure must be expected irrespective of treatment. In order to explore this possibility in a preliminary way, the further greenhouse experiment now described was undertaken.

Experimental

Elite seed of the variety O.A.C. 21, supplied by the Department of Field Crops, University of Alberta, was used. Two concentrations of indolylacetic acid, namely 2.5 and 5 p.p.m. by weight of the seed, were applied in a talc carrier dust at the rate of ½ oz. per bushel. The weighed quantity of grain to be treated was placed in a wide-mouthed glass vessel, the dust added, and the whole rotated mechanically for 10 min. to effect thorough dispersal. A control batch of seed, dusted with talc only, was prepared in the same way.

The experimental plants were grown in ordinary 5½ in. flower pots, each of which contained 1 kg. of steam-sterilized soil. Two seeds per pot were planted at a depth of 1 in., but subsequent thinning, by random selection, reduced the number of plants carried through to maturity to one per pot. Forty replicate pots were sown to each treatment, including the control. At the outset, all grew under the same conditions, water being added by weight every second or third day so as to bring the moisture content of the soil up to 30%, which was about half the moisture-holding capacity of 59%. As soon as germination was complete, however, the moisture supply of half the replicates of each treatment was restricted, being adjusted to 22.5% instead of 30% throughout the subsequent course of the experiment. The remaining replicates continued at the original 30% level.

The arrangement in the greenhouse was on two benches, one bearing all the pots adjusted to 22.5% soil moisture, and the other all those adjusted to 30%. Within each bench, the grouping was in 20 randomized blocks, each containing one representative of the three treatments. There were thus 20 replications of each combination of phytohormone dosage and soil moisture.

Air temperature was controlled thermostatically. At first a diurnal range of 40 to 55° F. was imposed. This was later increased to 45 to 60°, then to 50 to 70°, and, in the final stages of ripening, the temperature on bright sunny days reached a maximum of 80° or more.

Seeding took place on January 20, and harvesting on 14 and 15 June, 1939.

Results

The first seedlings emerged above ground on February 3, and Table I indicates the numbers observed (from 80 seeds of each treatment planted) on subsequent dates. At first, it seemed that there might be some effect of

TABLE I

COUNTS OF EMERGED SEEDLINGS
(FROM 80 SEEDS PLANTED)

Date	Indolylacetic acid dosage		
	0 p.p.m.	2½ p.p.m.	5 p.p.m.
4/2/39	21	26	35
6/2/39	60	47	55
8/2/39	77	77	77
10/2/39	79	79	80
13/2/39	80	80	80

treatment upon germination rate, but the later counts suggest that this was a chance result. Final germination was 100% in all cases.

Differential soil moisture supply was imposed on February 9 as described above, and maintained thereafter. Tillering was first noticed on March 9, proceeding as shown in Table II. The appearance of tillers (axillary shoots) in the groups of plants receiving 30% soil moisture was in general from two to three days in advance of that in the groups receiving only 22.5%. Furthermore, at 30% soil moisture, both concentrations of indolylacetic acid used accelerated the tillering process by about one day. There is some suggestion of a similar tendency in the results at 22.5% soil moisture, but here the effect is not nearly so pronounced. By March 21 practically all the plants, irrespective of treatment, had at least one axillary shoot.

Beginning on February 20, the height of the main tiller of each plant was measured at weekly intervals. The resulting treatment averages are shown in Table III. The first five sets of these averages are each deduced from

TABLE II

NUMBERS OF PLANTS (OUT OF 40) WITH AXILLARY SHOOTS

Date	Soil moisture, 22.5%				Soil moisture, 30%			
	Indolylacetic acid dosage			Total	Indolylacetic acid dosage			Total
	0 p.p.m.	2½ p.p.m.	5 p.p.m.		0 p.p.m.	2½ p.p.m.	5 p.p.m.	
9/3/39	0	1	0	1	3	7	6	16
10/3/39	1	3	1	5	7	15	14	36
11/3/39	4	5	3	12	10	20	20	50
12/3/39	9	15	15	39	22	30	30	82
13/3/39	15	18	22	55	22	33	33	88
14/3/39	26	32	30	88	35	35	39	109
15/3/39	32	36	36	104	36	38	40	114
16/3/39	34	36	36	106	40	38	40	118
17/3/39	35	37	37	109	40	38	40	118
19/3/39	39	40	38	117	40	38	40	118
20/3/39	39	40	38	117	40	38	40	118
21/3/39	39	40	39	118	40	39	40	119

40 replicate plants, and the remainder from 20, the number of plants having been reduced to one per pot on March 26. A significant difference between the mean height of all plants receiving 30% soil moisture and that of all those receiving 22.5% was soon apparent, and became progressively greater as development proceeded. It was not, however, until April 17 that any difference ascribable to phytohormone treatment manifested itself. At that date, the mean for all plants receiving indolylacetic grown at 22.5% soil moisture became slightly but significantly higher than the corresponding control value. This difference persisted until the cessation of elongation on May 1, when it was of the order of 3%. By this time the plants receiving 30% soil moisture were on the average about 20 cm. taller than those produced under the more restricted moisture supply, but on no occasion was any effect of chemical treatment on plant height detectable within this group.

TABLE III

AVERAGE HEIGHT OF MAIN TILLER (CM.) TO TIP OF TALLEST LEAF OR OF APICAL GLUME

Date	Soil moisture, 22.5%				Soil moisture, 30%			
	Indolylacetic acid dosage			Average	Indolylacetic acid dosage			Average
	0 p.p.m.	2½ p.p.m.	5 p.p.m.		0 p.p.m.	2½ p.p.m.	5 p.p.m.	
20/2/39	11.8	11.8	12.0	11.8	11.9	11.9	12.3	12.0
27/2/39	14.7	14.7	14.1	14.5	14.7	15.3	15.5	15.2
6/3/39	21.1	21.3	21.3	21.2	21.9	22.6	23.3	22.6
13/3/39	24.5	25.2	24.7	24.8	26.8	28.4	28.7	28.0
20/3/39	29.2	29.5	29.6	29.4	33.2	33.9	34.3	33.8
27/3/39	34.4	36.4	36.2	35.7	40.0	41.2	41.8	41.0
3/4/39	38.7	39.4	39.8	39.3	46.2	47.8	47.9	47.3
10/4/39	49.0	50.1	49.8	49.6	60.8	61.9	62.6	61.8
17/4/39	62.2	63.5*	63.8*	63.2	78.4	79.7	79.8	79.3
24/4/39	69.8	72.1*	72.2*	71.4	91.2	91.6	92.8	91.9
1/5/39	70.4	72.8*	72.5*	71.9	93.4	93.7	93.5	93.5
8/5/39	—	—	—	—	93.6	93.8	94.6	94.0

* *Significantly exceeds control.*

Emergence of heads began on April 16, and was completed in the main tillers of both moisture groups by April 25, with no indication of any treatment effect (Table IV). In the side tillers it continued intermittently until May 17. A total of 35 of the secondary shoots of the plants growing at 30% soil moisture produced heads, as compared with only four of those at 22.5%. Here again, however, there was no discernible effect of the indolylacetic acid applications.

Maturity was actually attained about a week earlier under the conditions of restricted moisture supply, but both crops were harvested at the same time, namely June 14 and 15. Air-dry weight of straw, weight of grain and number of kernels were determined for each plant individually, the treatment averages of these quantities being given in Table V. As would be expected, the productivity of the plants receiving the more liberal supply of soil moisture

TABLE IV

NUMBER OF HEADS FULLY EMERGED, I.E., BASAL NODE OF RACHIS ABOVE LIGULE OF TOP LEAF SHEATH

Date	Soil moisture, 22.5%				Soil moisture, 30%			
	Indolylacetic acid dosage			Total	Indolylacetic acid dosage			Total
	0 p.p.m.	2½ p.p.m.	5 p.p.m.		0 p.p.m.	2½ p.p.m.	5 p.p.m.	
16/4/39	1	0	0	1	0	2	1	3
17/4/39	2	1	1	4	1	3	2	6
18/4/39	3	3	2	8	5	8	5	18
19/4/39	7	10	5	22	13	17	19	49
20/4/39	9	14	10	33	16	17	20	53
21/4/39	14	16	16	46	17	18	20	55
22/4/39	18	19	19	56	17	20	20	57
23/4/39	18	20	20	58	18	20	20	58
24/4/39	19	20	20	59	19	20	20	59
25/4/39	20	20	20	60	20	20	20	60
27/4/39	20	20	20	60	22	22	21	65
29/4/39	20	20	20	60	23	23	23	69
1/5/39	20	20	20	60	23	23	24	70
3/5/39	20	20	20	60	24	24	26	74
3/5/39	20	20	20	60	25	25	27	77
7/5/39	20	20	20	60	25	26	28	79
10/5/39	20	20	21	61	30	28	31	89
12/5/39	20	21	22	63	32	29	33	94
17/5/39	21	21	22	64	33	29	33	95

was markedly the greater. Analyses of variance indicate that at 22.5% the weight of straw secured from the seed dusted with either 2.5 or 5 p.p.m. of indolylacetic acid was significantly greater, by about 10%, than that resulting from the control seed dusted with talc only. There was, however, no significant difference in the corresponding weights of grain. In the 30% moisture series, no effect of chemical treatment upon the weight of either grain or straw was demonstrable. The average number of kernels per plant likewise showed no significant treatment difference under either moisture regime.

TABLE V

YIELD OF STRAW AND GRAIN

Item	Soil moisture, 22.5%				Soil moisture, 30%			
	Indolylacetic acid dosage			Average	Indolylacetic acid dosage			Average
	0 p.p..m	2½ p.p.m.	5 p.p.m.		0 p.p.m.	2½ p.p.m.	5 p.p.m.	
Av. wt. of straw per plant, g.	1.04	1.13*	1.15*	1.11	1.83	1.73	1.87	1.81
Av. wt. of grain per plant, g.	1.87	1.94	1.93	1.91	2.96	2.64	2.81	2.80
Av. no. of kernels per plant	45	46	46	46	70	64	68	67

* *Significantly exceeds control.*

It cannot be said that the results of this experiment as a whole are at all striking. They do however provide some further evidence for the supposition that beneficial effects from the phytohormone dust treatment of seeds may be forthcoming only under certain limiting conditions, and that stimulation of growth or yield in the field should not be expected to be of general occurrence.

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AN ABERRANT STRAIN OF PUCCINIA HELIANTHI SCHW.[1]

BY A. M. BROWN[2]

Abstract

The aberrant strain of rust here discussed was first observed in haploid infections arising from germinating teliospores of the *tuberosus* strain of *Puccinia Helianthi*. The teliospores occurred on a single leaf of *Helianthus tuberosus*. Teliospores on several other leaves of the same collection were induced to germinate, but their sporidia produced only normal infections. This strain of rust differed from the parent strain in colour, pathogenicity, spore viability, and host range, and was parctically intersterile with it. It also differed in urediospore size, shape, and echinulation, and in ability to withstand warm temperatures. It is suggested that these differences were probably due to a mutation in which the loss or gain of chromatin carrying more than one gene was involved.

Introduction

Owing to the intensive study to which certain of the plant rusts have been subjected in recent years, opportunity has been given for observing changes and aberrancies that might have otherwise passed unnoticed. For example, in *Puccinia graminis Tritici* Erikss. and Henn., Newton and Johnson (5) and Waterhouse (10) discovered spontaneous changes in spore colour, and Stakman, Levine, and Cotter (9), and Newton and Johnson (6), spontaneous changes in pathogenicity. All these changes were attributed to mutations. In *Puccinia triticina* Erikss., Johnston (4) reported an aberrant race differing from known races in length of incubation period, spore colour, and size of urediospores, and Roberts (8) described a mutation for pathogenicity. Gassner and Straib (3) recorded a mutation for pathogenicity in *Puccinia glumarum Tritici* (Schmidt) Erikss. and Henn., and d'Oliveira (7), one for colour and pathogenicity in *Puccinia anomala* Rostr.

For some years *P. Helianthi* has been studied at this laboratory, where it was shown to be heterothallic (2) and to comprise several well defined strains (1), one of which is specialized to *Helianthus tuberosus* L. and known as the *tuberosus* strain. Early in 1936, monosporidial infections of this strain were under observation, and among them were detected occasionally infections that were whitish in colour but otherwise apparently normal. These aberrant infections produced hyaline paraphyses and pycniospores, and colourless nectar. As a rule they failed to attain the diameter of the normal infections in the same cultures.

This particular strain of *Puccinia Helianthi*, as already reported (1), was collected on an isolated patch of *Helianthus tuberosus* growing near the Red River. The collection was made from the same clump of plants from which collections had been made in previous years. Pathogenically the strain

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seemed to be homozygous, for cultures of it had been selfed yearly during the five preceding years and each year the same pathogenic strain was isolated. The telial material used as a source of inoculum for the present study was collected early in November, 1935. The usual inoculation procedure was followed. A leaf bearing numerous telia was randomly selected, soaked in water, and dried several times in succession, and, when sporidial production became abundant, the leaf was suspended over seedling plants of *Helianthus annuus* L.

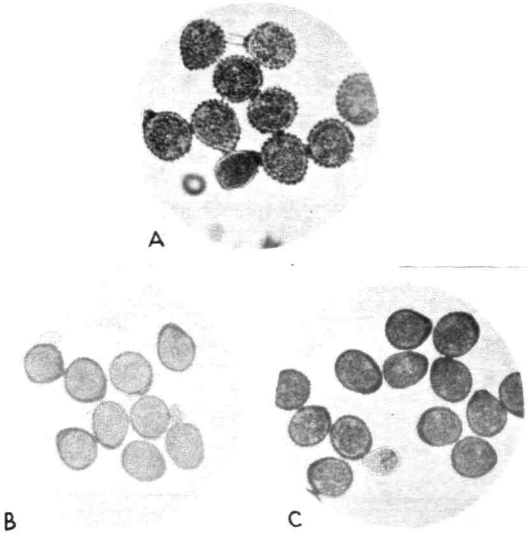
From the telia of the first leaf used for inoculation purposes there arose a number of haploid infections among which were three that were whitish. These three occurred haphazardly among, but well isolated from the other infections. When they were 21 days old, their scantily produced colourless nectar, resembling drops of glycerine, was intermixed, each one of the three infections receiving nectar from the other two. Fortunately the three haploid infections were not all of the same sex, and several days after the nectra transfer they all produced whitish aecia and aeciospores. These aeciospores germinated less vigorously than did those produced concurrently in normal infections similarly selfed. When sown on seedling hosts, they produced uredia and urediospores, both greyish.

As the leaf bearing the telia was not discarded after the first inoculation, it was used at two-week intervals for further inoculations, until teliospore germination failed towards the end of March. In each set of cultures established from the telia on this leaf, occasional white infections appeared. Altogether, 10 additional ones were obtained that were well separated from any other infections, and in these, too, aecia were induced to develop. In mass, the aeciospores, like those just referred to above, were whitish and gave rise to infections that produced greyish-coloured urediospores and teliospores. Although teliospores on several other leaves of the same collection were induced to germinate, their sporidia produced only normal infections.

Comparison of Normal and Aberrant Urediospores

The aberrant urediospores, as can be seen in Plate I, B, are readily distinguishable from those of the normal *tuberosus* strain, Plate I, A, on account of their pale appearance. Moreover, they are smoother and smaller than those of that strain. By projecting on a screen the images of 100 urediospores from each of two different generations of the aberrant and of the *tuberosus* strain at a magnification of 500, the spore sizes given in Table I were secured. For comparative purposes, similar measurements were obtained for urediospores of the *annuus* strain, a strain apparently capable of attacking only *Helianthus annuus*. From these data it is evident that there is a distinct difference between the size of the urediospore of the aberrant and that of the urediospore of the normal strains. In the two generations referred to, the urediospores of the *tuberosus* strain were consistently larger than the urediospores of the aberrant or of the *annuus* strain, those of the two latter strains being much the same in size.

PLATE I



A. Urediospores of the tuberosus strain. They are very rough in outline. $\times 360$. *B. Urediospores of the aberrant strain. Owing to their greyish colour, these spores appear somewhat hazy in the photograph.* $\times 360$. *C. Urediospores of the annuus strain.* $\times 360$.

TABLE I

THE MEAN LENGTH AND WIDTH OF UREDIOSPORES AND THE STANDARD ERROR OF MEAN, FOR TWO GENERATIONS OF SPORES

	Mean spore length in μ	Mean spore width in μ
1st urediospore generation		
Tuberosus strain	28.8 ± 0.013	25.5 ± 0.011
Annuus strain	24.9 ± 0.010	21.6 ± 0.007
Aberrant strain	23.7 ± 0.011	19.7 ± 0.011
2nd urediospore generation		
Tuberosus strain	29.6 ± 0.048	25.6 ± 0.048
Annuus strain	24.5 ± 0.014	21.4 ± 0.007
Aberrant	24.5 ± 0.010	21.1 ± 0.013

These differences in spore character are further shown in Plate I, which shows urediospores of the three strains photographed at an identical magnification. The spores of the normal *tuberosus* strain show pronounced echinulation, while those of the other two strains are more finely echinulate.

Pathogenicity of the Three Strains

In a previous investigation (1), the *tuberosus* and *annuus* strains proved to be quite dissimilar in host range. For the purpose of comparing the pathogenicity of the aberrant strain with that of each of the other two strains, the following hosts were inoculated with urediospores of each of the three strains: *Helianthus annuus*, *H. subrhomboideus* Rydb., *H. Maximiliani* Schrad., *H. subtuberosus* Bourg., and *H. tuberosus*. All these hosts were raised from seed, except *H. tuberosus*, which grew from tubers procured from the location at which the telial material of the *tuberosus* strain was collected.

The results of this experiment, shown in Table II, indicate that the three strains differ in their pathogenic capabilities. The aberrant strain, although derived from the same telial material as the normal *tuberosus* strain differed from it in host range and pathogenicity. It attacked *Helianthus tuberosus* only weakly and *H. subtuberosus* and *H. subrhomboideus* not at all, whereas

TABLE II

THE REACTIONS OF FIVE SPECIES OF *Helianthus* TO UREDIOSPORE INOCULATION OF THE *tuberosus*, *annuus*, AND ABERRANT STRAINS

Source of urediospore inoculum	*H. annuus*	*H. subrhomboideus*	*H. maximiliani*	*H. subtuberosus*	*H. tuberosus*
Tuberosus strain	S*	S*	O	S	S
Annuus strain	S	O	O	O	O
Aberrant strain	S	O	O	R	R

S = *susceptible*; R = *resistant, with minute infections*; O = *immune*.
* *Uredia produced on upper leaf-surface only.*

the normal strain attacked all three vigorously. Like the *tuberosus* strain, it infected readily *Helianthus annuus*. the only one of the five hosts attacked by the *annuus* strain. It was less tolerant of warm temperatures than either of the other two strains, for, during the ensuing summer, the aberrant strain became progressively weaker and was finally lost.

Interfertility of the Three Strains

To test the interfertility of the three strains, urediospores of the aberrant strain were sown near to the periphery of 100 monosporidial infections of the *tuberosus* and of 30 similar infections of the *annuus* strains. As a control, aberrant urediospores were sown near the periphery of six aberrant infections. The results of this experiment are shown in Table III. Briefly they show that, 12 days after the inoculation, all the uredial infections had coalesced with the haploid infections, and, at the end of 21 days, three infections of the *tuberosus*, four of the *annuus*, and the six aberrant ones produced aecia.

TABLE III

THE RESULTS OF PAIRING HAPLOID INFECTIONS OF THE *tuberosus*, *annuus*, AND ABERRANT STRAINS, WITH UREDINAL INFECTIONS OF THE ABERRANT STRAINS

Haploid infections	Uredinal infections	No. of haploid infections	No. of infections with aecia
Tuberosus strain	Aberrant strain	100	3*
Annuus strain	Aberrant strain	30	4
Aberrant strain	Aberrant strain	6	6

* *Aeciospores were non-viable.*

The aecia and aeciospores produced by the diploidised infections of the *tuberosus* and *annuus* strains were normal in colour, while those of the aberrant infections were whitish and in every respect similar to the aecia and aeciospores induced earlier in aberrant infections by selfing. In the diploidised infections of the *tuberosus* and *annuus* strains, aecia were sparsely produced and the aeciospores of the former strain failed to germinate. Those of the *annuus* strain, however, showed a trace of germination, although, when sown on *Helianthus annuus* seedlings, they failed to cause infection. On the other hand, aeciospores from the diploidised aberrant infections germinated moderately well and, when sown on *H. annuus* seedlings, produced greyish uredia and urediospores. These urediospores were identical in infection capabilities with those first obtained of the aberrant strain.

Discussion

How this strain of rust originated must remain at least partly a matter of conjecture. During the five years preceding and the three years following the isolation of this aberrant strain, cultures were established from telial

material collected from the same clump of plants as that from which the present telial material was obtained. Although numerous infections were procured in each of those years, no aberrant ones were observed among them. The fact that the sporidia from which the aberrant haploid infections arose were produced only by teliospores from one particular leaf suggests that the mycelium of some telial sorus on that leaf carried a chromosomal irregularity that was responsible for the aberrancies. As already shown, this strain differed from the normal strain in colour, pathogenicity, spore viability, and host range, and was practically intersterile with that strain. It differed also in urediospore size, shape, and echinulation, and in ability to withstand warm temperatures. These differences were probably due to mutation, but, in view of the number of characters involved, it seems unlikely that a single gene, or "point", mutation alone was responsible for them. Rather would it appear that the change involved the loss or gain of chromatin carrying more than one gene.

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REPRODUCTION IN SOME POA SPECIES[1]

By V. Engelbert[2]

Abstract

The writer has demonstrated through breeding experiments repeated over three years, that *Poa arctica* R. Br., *Poa alpigena* Fr. Lindm., *Poa alpina* L. from Greenland as well as *Poa alpina* L. from Georgian Bay, Ontario, and a race of *Poa pratensis* L. from Gaspé, Quebec, are all apomictic (parthenogenic) and pseudogamous. In pseudogamy the pollen tube enters the stigma and activates the apomictic embryo sac to development, but it does not as a rule enter the ovarian tissue to achieve fertilization of the ovule. Pollen from any one species germinated readily and with a high percentage on the stigmas of any one of the others. Pollen sterility did not exceed 2%.

Introduction

A number of Scandinavian workers have investigated some phases of reproduction in this group of grasses. On the basis of cytological and breeding experiments Müntzing (5) suggested in 1932 that certain races of *Poa alpina* L. and *Poa pratensis* L. were apomictic in their method of reproduction. This was the first demonstration of apomixis in the Gramineae. Aakerberg (1) confirmed this report after breeding experiments with different races of the same species. He also found pseudogamy as a rule, but out of several thousand attempted crosses he (2) succeeded in obtaining a few hybrids of *P. pratensis* × *P. alpina* and between different *pratensis* plants. He has since (3) published further observations on apomictic and sexual seed formation in *Poa pratensis*. Kiellander (4) found apomixis also in *Poa serotina* Ehr.

Experimental

Material:

The species used were:

Poa arctica R. Br. from west Greenland.
Poa alpina L. from west Greenland.
Poa alpigena Fr. Lindm. from west Greenland.
Poa alpina L. from Georgian Bay, Ontario.
Poa pratensis L., a biotype from Gaspé Peninsula, Quebec.

The species from Greenland were grown from seed obtained through the kindness of Mr. M. P. Porsild, Director of the Danish Arctic Station on Disko Island, Greenland. The Canadian material was collected by the writer in 1935 as both seed and as vegetative clones.

Even with greenhouse facilities and a longer day produced by electric light, it takes two years to rear a plant of these species to the flowering stage suitable for experiment.

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Breeding Experiments

I. Method

The flowers of the Poas are so small as to require the development of a special technique for emasculation and pollination. To provide sufficient magnification a 30× Leitz binocular microscope was used. The panicle was stuck through the ring of a retort stand while its flowers were being emasculated. While performing this operation the hands rested on the ring so that the necessary steadiness of movement and reasonable efficiency and speed were ensured. The floret was held lightly between the forefinger and thumb of the left hand. A pair of surgeon's "nerve forceps" with curved points were used both for opening the flower by displacing the lemma or palea and removal of the anthers. As soon as the stigmas showed their feathery branches outside the glumes, pollinating was begun. To obtain the pollen a clean glass slide was tapped from below against the ripe protruding anthers. The pollen would then cover the slide as a fine dust and its quality could be observed quickly under a compound microscope. If the quality were satisfactory, the slide was passed gently over the stigma branches of the mother plant. This method ensured that an abundance of pollen was applied without injury to it or the stigmas.

It was very important that the pollen be taken as soon as the anthers showed outside the glumes owing to its extreme sensitivity to such external factors as temperature and the relative humidity of the air. The following observations apply to all the species used and to a location in Ontario at approximately 43½° N. Lat.

As the atmosphere becomes drier and warmer through May and June, the life of the pollen grain becomes progressively shorter. About May 20, anthesis usually takes place between 8 and 10 a.m. At this time sound, undeteriorated pollen can be obtained until noon and on a cool cloudy day over the first part of the afternoon. As the weather gets warmer and drier anthesis takes place much earlier and virtually all the pollen has shrivelled by 6 a.m. on a bright warm morning. On a dull day, anthesis will be delayed until afternoon even in June. Every sample of pollen used for pollination was therefore examined under the microscope to ensure that only sound pollen was applied to the stigmas. This rapid deterioration of the pollen of *Poa* seems never to have been mentioned by other workers. Shrivelled pollen gathers in lumps on the slide when shaken out of the anther but the sound pollen forms a fine dust-like layer.

Aakerberg (1, 2) from his observations classes the pollen as (i) well developed, morphologically good, (ii) not quite full of plasma, or (iii) empty pollen. The writer suggests that (ii) is partly deteriorated pollen and that probably most of the pollen mentioned under (iii) can be classed as completely deteriorated pollen. Very little pollen that was truly sterile was found in the species here studied, its frequency being at the most 1 to 2%. The sterile pollen appears yellowish, whereas the deteriorate pollen is whitish, and thus they can easily be distinguished from each other.

II. Results

A. Breeding Data

The series of experiments here reported were performed in 1936 and repeated in 1937 and again in 1938 with uniformly consistent outcome. The mode of reproduction and the possibilities of natural and artificial hybridization were tested with five different treatments.

(*a*) Emasculation without subsequent pollination; 1282 flowers were so treated and no seed was produced in any instance.

(*b*) Emasculation followed by artificial self-pollination; 1049 flowers were treated and from 12 to 30% of them produced seed. No female sterile plant was found.

(*c*) Emasculation and interspecific cross-pollination; 1549 flowers were treated and from 1.4 to 59% of the flowers set seed. No sterility was found here either. All the progeny resembled closely the mother plant.

(*d*) Control plants left to natural development but covered with bags produced from 3 to 55 seeds per 100 flowers.

(*e*) Control plants not covered with bags and left to strictly natural development produced from 13 to 77 seeds per 100 flowers.

The great variation in seed production in isolated plants is due partly to the bagging and partly to climatic conditions. The latter influence is seen in the seed production of control plants not bagged. There is no evidence that any one of these species or any single plant is an especially good or poor seed-producer.

From these results it is evident that pollination is necessary for seed production but that the progeny all resemble the mother parent. The pollen then must act as an activator on the ovule, but actual fertilization does not take place; this is pseudogamy, a type of apomixis.

B. Pollen Germination Studies

The germination of pollen was studied and followed microscopically by mounting whole stigmas in lactic phenol and methyl blue at from 2 to 48 hr. after pollination, with two hour intervals the first day.

The pollen germination was high in all cases whether it belonged to the same or a different species from that of the mother plant. The pollen tubes remained short, having an average length of 2 to 4 pollen grain diameters. Only one exception was found, in self-pollinated stigmas of one plant of *Poa alpina*, where the pollen tubes reached the ovarian tissue. The initial growth of the pollen tubes is quite rapid. Stigmas pollinated about 5 a.m. showed many pollen tubes ranging from one to two pollen grain diameters about 9 a.m. to a maximum of five to eight at 12.00 a.m.

There are, no doubt, differences in the speed of pollen tube growth and in the ultimate length attained depending on the condition of the stigma and of the pollen at the time of application as well as the climatic factors at the

time. Thirty-six hours after pollination the ovaries had increased considerably in size in all specimens examined. These results support the conclusions drawn from the breeding experiments and show that the pollen tube only acts as an activator and not in a genetic capacity.

Conclusions

The species investigated show both from breeding experiments and pollen germination studies that they are apomictic and pseudogamous.

Before the taxonomy of the Poas can be fully outlined and the speciation process properly analysed, it is necessary to know the method of reproduction of the different species and their various geographic races.

The *Poa* species so far investigated are characteristically highly polymorphic. Being preponderantly pseudogamous, these species and their sexual relatives must naturally be given different ratings. From the standpoint of forage crop plant breeding it is important that apomixis can be demonstrated as a mode of reproduction in economically useful species such as *Poa pratensis* L.

The progeny of apomictic or pseudogamous types really are "clones" and the initial selection can also be the final one. One must expect to find both apomictic and sexual races within the one species as well as rare cases of sexual seed-production in the apomictic ones.

An investigation of the development of the male and female gametophyte in the species used here is in progress.

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A ROOT-ROT OF DOUGLAS FIR CAUSED BY *PORIA WEIRII*[1]

By Irene Mounce[2], J. E. Bier[3], and Mildred K. Nobles[4]

Abstract

A laminated root-rot of Douglas fir [*Pseudotsuga taxifolia* (Lamb.) Britt.] occurs in young stands on Vancouver Island, B.C. The decay, the sporophore associated with it, and the cultural characters of the fungus are described. A comparison with *Poria Weirii* Murr., previously reported only on *Thuja plicata* (D. Don.), shows a close resemblance in all respects between the two fungi.

During the last few years foresters at Cowichan Lake Forest Experiment Station have observed the dying in groups of young Douglas fir trees. The frequent occurrence of such affected areas showed that the trouble was of considerable importance in young second growth stands. Their general appearance suggested at first that *Armillaria* root-rot might be responsible. Recent investigations, however, indicate that, while that disease is present, most of the damage is due to a species of brown *Poria*. Although differing somewhat from *Poria Weirii* Murr. as it is found on western red cedar (*Thuja plicata* D. Don.), the fungus would seem to be that species or a form of it occurring on Douglas fir.

The Disease

Our first record of this disease dates back to November 30, 1929, when Mr. C. G. Riley who was then with the British Columbia Forest Branch, Victoria, sent in for identification two specimens of decay in Douglas fir and the cultures made from them. Both were from the Cowichan Lake Forest Experiment Station. One wood specimen (No. 1277) was taken from the base of a living Douglas fir growing in a pure 18-year-old stand. The discoloration of the heartwood ran up the stem to a height of 3 ft. No fruit-bodies were found. The other specimen (No. 1278) showed mycelial fans beneath the bark. "The fungus appears to be responsible for some serious gaps in an otherwise thrifty stand of Douglas fir twenty years old. Its habit resembles *Armillaria mellea* but the fruit-bodies, if of that species, are atypical." The culture of the first organism is identical with isolates made during the present study, the second is, as Mr. Riley surmised, *A. mellea*.

Geographical Distribution

Observations made so far indicate that this disease occurs generally throughout the southeastern portion of Vancouver Island (Fig. 1). Collections have been made from Saanichton, Duncan, Cowichan Lake, Nanaimo, Parksville,

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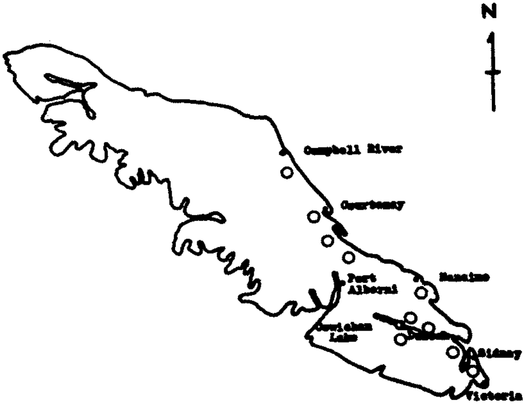


FIG. 1. *Localities (○) on Vancouver Island, B.C. where root-rot of Pseudotsuga is known to occur.*

Union Bay, Cumberland, and Oyster River. It is not known to be present on the mainland but to date the survey has been limited to the examination of a number of young stands of Douglas fir in the Fraser Valley between Vancouver and Mission. More extensive surveys are to be made during the summer of 1940.

Hosts and Occurrence

Critical field observations on the disease were made in stands located at the Cowichan Lake Forest Experiment Station. Infection was common in 20-, 30-, and 40-year-old Douglas fir and in addition the pathogen has been isolated from rot in 100- and 200-year-old Douglas fir (Plate II, Fig. 2) as well as from western hemlock [*Tsuga heterophylla* (Raf.) Sarg.]. The importance of the disease in older stands of fir and on hemlock is not known at this time.

To determine the incidence of the disease a survey was made of the 35- to 40-year-old Douglas fir occurring in the North Arm area (west of road) of the Station, a location that may be considered as representative of the region. Fig. 2 illustrates the frequency of the infection centres, each of which has a number of diseased and recently killed trees. Although the disease mainly occurred in patches it was not uncommon to find isolated trees that were infected.

Five one-tenth-acre sample plots were established in areas designated as infection centres in Fig. 2 to obtain further information on the number and crown class of the trees killed. A summary of this study is given in Table I.

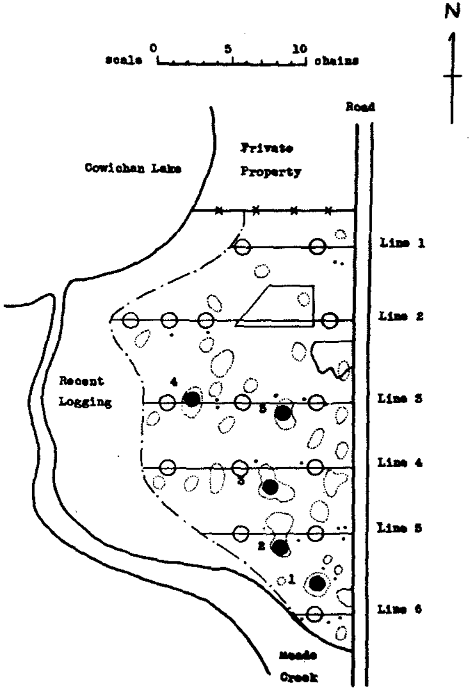


FIG. 2. *Incidence of root-rot in Pseudotsuga in the North Arm area (west of road) of the Cowichan Lake Forest Experiment Station, B.C.*
(○) *Centres of infection.*
(·) *Isolated trees killed during 1939.*
(●) *Location of disease plots.*

It is evident that from 32 to 57% of the trees on the plots have been killed by root-rot. Further, from 19 to 40% of the dominants and co-dominants were affected; this demonstrated that the disease is not confined to the less vigorous trees of the poorer crown classes. It is well to remember that the above figures apply to the infection centres shown in Fig. 2, and not to the stand as a whole.

Figures for Permanent Plot 200 of the British Columbia Forest Service are also included in Table I. All living Douglas firs were tagged in 1935, and,

TABLE I

INCIDENCE OF ROOT-ROT IN DOUGLAS FIR IN THE NORTH ARM AREA (WEST OF ROAD) OF THE COWICHAN LAKE FOREST EXPERIMENT STATION, VANCOUVER ISLAND, B.C.

Plot No.	Number of trees on plot	Number killed by root-rot	Per cent killed by root-rot	Number of dominants and co-dominants on plot	Number of dominants and co-dominants killed by root-rot	Per cent of dominants and co-dominants killed by root-rot
1	34	17	50	22	8	36
2	58	25	43	21	5	24
3	48	25	52	21	4	19
4	19	11	57	5	2	40
5	34	11	32	14	5	36
200	41	10	24	29	8	28

consequently, dead trees which still bear tags have been killed during the last four years. The table shows that during this interval 10 of the 41 tagged trees, i.e., 24% have been killed as the result of root-rot. Eight of the ten trees killed were classified as dominants and co-dominants in 1935.

In most instances trees are killed in groups; this results in stand openings that appear to increase in size gradually. This is illustrated in Fig. 3, which represents diagrammatically one of the permanent sample plots established to obtain further information on the spread of the disease. Wind and snow damage is common and it is possible that the stand openings resulting from root-rot are indirectly responsible for this injury (Plate II, Fig. 1).

Appearance

The first indication of the presence of the disease is a retardation of growth. Infection in the roots interferes with food supply and reduces the current season's growth (Plate I). This is usually accompanied by a "distress" crop of cones, which are smaller than normal. An infected tree may show these signs, accompanied by a gradual thinning of the foliage, for a year or two before there is a browning of all the needles, signifying that the tree is dead. Because of the decayed roots these trees are subject to wind and snow damage and they are commonly found uprooted with the large roots broken off a short distance from the crown (Plate II, Fig. 1). Baxter (1, p. 331) has described a similar condition in stands of *Thuja plicata* attacked by *Poria Weirii*.

Decay

Incipient decay.—A brownish stain characteristic of the incipient stage of decay is usually present on the transverse face of the stump of a recently killed tree (Plate III, Figs. 1–4). The affected area is crescent-shaped to irregular in outline and principally confined to the heartwood, though with frequent patches extending into the sapwood. The discoloration has been

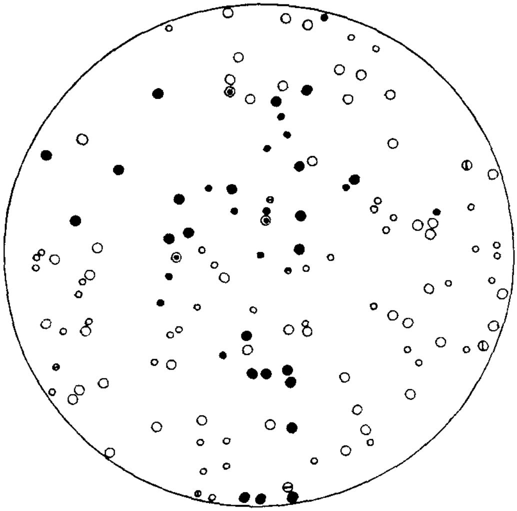


FIG. 3. *Permanent Sample Plot 2, Cowichan Lake Forest Experiment Station, B.C. 1939. Area 0.25 acres.*

(○) *Dominants and Co-dominants.*
(○) *Intermediate and Suppressed.*
(○) *Pseudotsuga taxifolia.*
(⊖) *Tsuga heterophylla.*
(⦶) *Abies grandis.*
(⊙) *Thuja plicata.*
(●) *Dead, owing to root-rot.*

found for a distance of from 4 to 6 ft. in advance of the later stage. Occasionally there is no rot apparent in the stem above ground and it is found only by exposing the roots.

Advanced decay.—On the transverse face of a stump the advanced decay appears at first as small separated areas. If such a stump is split it is possible to trace each of these areas of rot back to individual decayed roots. Later the entire central core of the stump is rotted, and in a few instances this core of advanced decay has been found to extend for some distance up the trunk of living trees (Plate III, Figs. 5, 6).

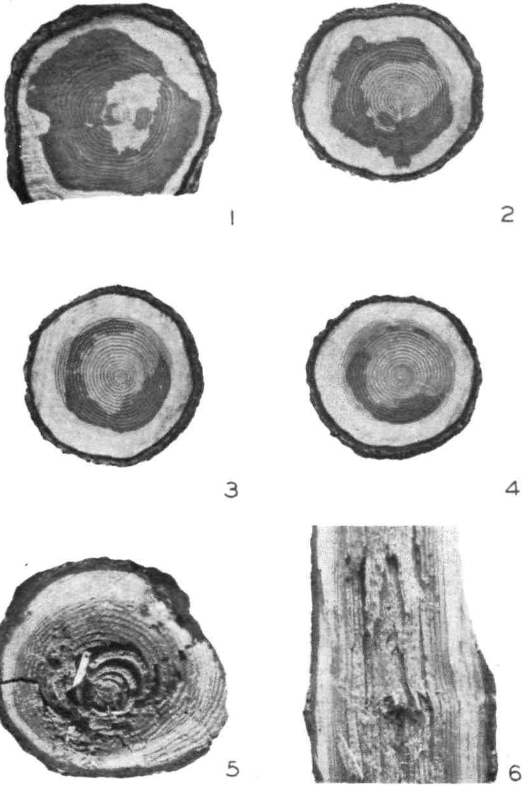
In the later stages the wood is soft and flaky, yellowish to brownish, and "honeycombed with small pockets which at first may be filled with whitish fibres but later the pockets are empty." The annual rings separate readily to form a typical ring-scale or laminated rot (Plate III, fig. 5). Tufts of mycelium,

PLATE I

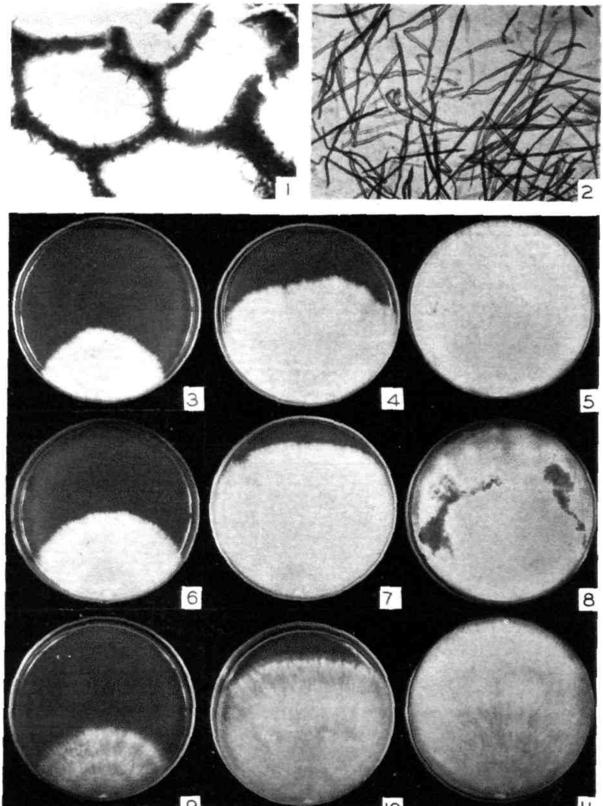
Poria Weirii root-rot in a 20-year-old stand of Pseudotsuga taxifolia at Cowichan Lake Forest Experiment Station, B.C. On the left a diseased tree with thin foliage, in the centre an infected tree with short current growth and "distress" crop of cones, on the right healthy trees.

FIG. 1. *Three infected trees uprooted by wind showing the roots broken off at a short distance from the crown.* FIG. 2. *Saprot produced by Poria Weirii in 200-year-old Pseudotsuga at Cowichan Lake Forest Experiment Station, B.C.* FIG. 3. *Young fruit-bodies of Poria Weirii on a stump of Pseudotsuga.* FIG. 4. *Older fruit-bodies on the under side of a stump of Pseudotsuga.*

PLATE III



FIGS. 1 - 4. *Typical appearance of the decay in a 35-year-old stand of Pseudotsuga at Cowichan Lake Forest Experiment Station, B.C.* FIG. 1. *At ground level.* FIG. 2. *Eighteen inches above the ground.* FIG. 3. *Three feet above the ground.* FIG. 4. *Four and one-half feet above the ground.* FIGS. 5, 6. *Decay at breast height in the trunk of a living Pseudotsuga showing the pitted and laminate character of the advanced rot.*



FIGS. 1–8. *Poria Weirii from Pseudotsuga.* FIG. 1. *Cross section of pores of fruit-body, showing setae.* ×*50 approx.* FIG. 2. *Setal hyphae from aerial mycelium of culture.* ×*120 approx.* FIGS. 3–5. *Culture from fruit-body tissue, photographed after one, two, and four weeks respectively.* FIGS. 6–8. *Culture from rot, photographed after one, two, and four weeks respectively.* FIGS. 9–11. *Poria Weirii from Thuja plicata. Culture from rot below fruit-body, photographed after one, two, and four weeks respectively.*

which may coalesce to form sheets, avellaneous and wood brown to army and Natal brown in colour (6) are present in the advanced decay. This mycelium is also found on the outer surface of the bark of diseased roots and on the soil immediately surrounding them. Brown setal hyphae are abundant and readily visible when the mycelium is examined with a hand lens.

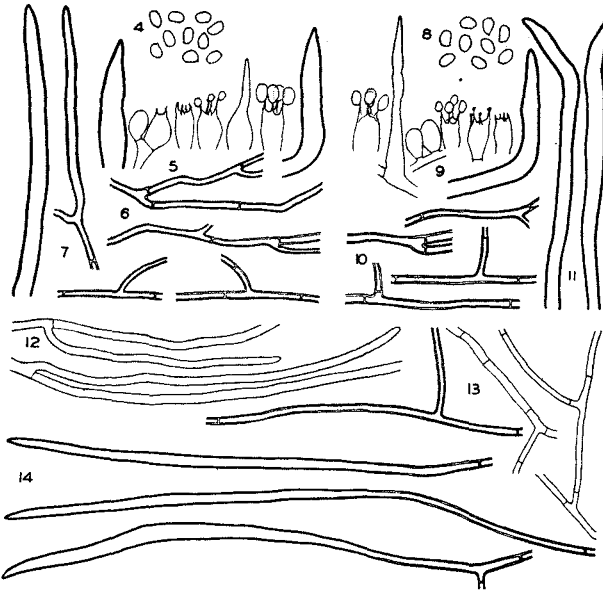
With the exception of the paler avellaneous colour sometimes found in the mycelial mats this rot in Douglas fir does not differ from that caused by *Poria Weirii* in western red cedar. In both, the decay occurs in living trees, is confined largely to the roots and butt, and consequently is indirectly responsible for losses caused by windfall, the affected wood is pitted, the annual rings separate readily, and tufts of mycelium containing setal hyphae are present between the layers. Finally, the sporophores associated with the rots appear to be identical.

The Fungus

Sporophores

In late summer and early autumn, fruit-bodies develop abundantly on the underside of logs, uprooted stumps, and occasionally on the trunks of dead standing trees of *Pseudotsuga* (Plate II, Figs. 3, 4). This fungus, like *Poria Weirii on Thuja* (1, p. 331), continues to grow after the tree has been killed, and the sporophores are not found as a rule until the wood is in an advanced stage of decay, i.e., on trees that have been dead for some time.

Sporophores are perennial, light in weight and soft and fragile; effused for a metre or more, separating rather readily from the substratum. In young developing fruit-bodies there is sometimes a broad, sterile margin (Plate II, Fig. 3) cinnamon-buff to pinkish buff and cinnamon later becoming Sudan brown (6); the context is 1 mm. thick, ochraceous, sayal brown to cinnamon-brown; the pore layer 4 mm. thick, the pores somewhat irregular in shape, thin-walled, 4–5 per mm., tawny-olive to Saccardo's umber and Brussels brown, occasionally with the purplish tones of army brown and Natal brown. In older specimens the pore layer is 6–10 mm. thick, deeply cracked, pores 5–6 per mm., the walls thin and entire, uniformly snuff brown, Verona brown or warm sepia (Plate II, Fig. 4). The spores are smooth, hyaline, broadly ellipsoid, becoming oblong-ellipsoid, apiculate at maturity, 4–4.9 × 2.8–3.2 μ (Fig. 8); setae abundant (Fig. 11; Plate IV, Fig. 1), pointed, occasionally encrusted, 4–10 μ diameter projecting up to 30 μ, actually the projecting ends of embedded setal hyphae, which are numerous in subiculum and trama (Fig. 11); cystidia (?) hyaline, with bulbous bases and attenuated tips, similar to those in *Poria punctata*, as described by Overholts (5). They appear to be aberrant basidia (Fig. 9). Hyphae of the subiculum are 3.0–4.5 μ diameter, brown, with walls thin or slightly thickened and simple septa, sparingly branched, with, as a rule, "the branches originating at a point median between two adjacent cross-walls," as noted by Overholts (5) in *Poria Weirii* on *Thuja*, but occasionally with branches originating below septa. Hyphae of the trama similar, except that the branches usually originate below septa and rarely midway between septa (Fig. 10).



FIGS. 4 – 7. *Poria Weirii on Thuja plicata.* FIG. 4. *Basidiospores.* ×*800.* FIG. 5. *Setae, cystidium, basidia and basidiospores.* ×*800.* FIG. 6. *Hyphae from context and trama.* ×*450.* FIG. 7. *Setal hyphae.* ×*450.* FIGS. 8 – 14. *Poria Weirii on Pseudotsuga taxifolia.* FIG. 8. *Basidiospores.* ×*800.* FIG. 9. *Seta, cystidium, basidia, basidiospores.* ×*800.* FIG. 10. *Hyphae from context and trama.* ×*450.* FIG. 11. *Setal hyphae.* ×*450.* FIG. 12. *Hyaline hyphae from advancing zone of culture.* ×*450.* FIG. 13. *Coloured hyphae from aerial mycelium of culture.* ×*450.* FIG. 14. *Setal hyphae from aerial mycelium of culture.* ×*450.*

Murrill (4) in his original description of the species gave the spores of *P. Weirii* as "ellipsoid, smooth, hyaline, 5 × 3 μ"; Overholts (5) described them as "globose to subglobose, smooth, hyaline, 4–6 μ diameter". The young sporophores on Douglas fir (Plate II, Fig. 3) are in excellent condition with an abundance of basidia and spores. The spores attached to basidia are globose to subglobose at first but become broadly ellipsoid and finally oblong-ellipsoid with a small apiculus, 4–4.9 × 2.8–3.2 μ (Fig. 8). Although we have specimens of *P. Weirii* on *Thuja* from Bovill and Harvard, Idaho; Marysville, Wash.; Rosebery, Lumby, Robert's Creek, and West Vancouver, B.C., spores were found only in the Rosebery collection. They agreed in size and shape with those from *Pseudotsuga* collections, i.e., young spores

were globose, later becoming oblong-ellipsoid and measuring 3.9–4.9 × 2.7–3.6 μ (Fig. 4).

The sporophores from *Pseudotsuga* do not differ in any fundamental character from *Poria Weirii* on *Thuja* as described by Murrill (4) and later by Overholts (5). The latter fungus may persist for five or six seasons and become 2 cm. thick, whereas so far no specimens of the former have been found to persist for more than two or at most three seasons—perhaps because they are destroyed by insects. Similarly fruit-bodies from the two hosts are identical in their microscopic characters, including size and shape of basidia, basidiospores, cystidia (?), and hyphae (Figs. 4–7 and 8–11). From our measurements the average diameter of the setae in specimens from *Thuja* seems slightly greater than that in specimens from *Pseudotsuga*, the range being 6–13.5 μ (Overholts 6–12 μ) as compared to 4–10 μ. In cultures however the reverse seems to be the case: isolates from *Pseudotsuga* gave setae 4.5–6 (–7) μ while those from *Thuja* were 3–5 μ in diameter. Hence variations in this character are probably not significant.

In any case, the sporophore with its colour, texture, light weight, cracking in older specimens, abundant setae and setal hyphae, and the hyaline, ellipsoid to oblong-ellipsoid spores, the peculiar mode of branching of the hyphae of the subiculum, together with the production of a pitted laminated root- and butt-rot would seem to preclude this fungus on *Pseudotsuga* from being anything but *P. Weirii* or a variety of it.*

It is interesting to note that Korstian and Brush (3) report that in Southern white cedar [*Chamaecyparis thyoides* (L.) B.S.P.] "an unidentified, laminated spongy butt rot is sometimes found, but it has not been possible to connect it with the sporophores of any fungus. In appearance the rot resembles that caused by *Poria Weirii* on western red cedar and may eventually be found to be the same." This suggestion, coupled with our records of its occurrence on *Pseudotsuga* and *Tsuga*, shows that the host range of *Poria Weirii* is wider than previous reports would indicate.

Cultures

Thirty-six isolations of the causal fungus were made from the sources listed in Table II. All the isolates are similar and readily recognizable.

To determine the cultural characters of the fungus and compare them with those of *Poria Weirii* from *Thuja*, 10 isolates of the former and two of the latter were examined according to the procedure used in this laboratory. The cultures are grown on 2% Difco malt agar in 9-cm. Petri dishes in the dark at room temperature, and examined at weekly intervals for six weeks. Observations are made on (i) rate of growth as measured by radius of colony from the inoculum which is placed at the edge of the Petri dish, (ii) colour based on Ridgway's standards, (iii) character of margin, (iv) contour and texture of mat, (v) colour changes in the agar ("Reverse"), and (vi) odour.

**It was not possible to compare the sporophores from Douglas fir with the type material of Poria Weirii since the latter is not available for study just now.*

TABLE II

ISOLATIONS OF THE PATHOGEN

No. of isolations	Host	Source
2	*Pseudotsuga taxifolia*	Stained wood in roots; foliage thin but still green
26	*Pseudotsuga taxifolia*	Rot in roots or base of young living or recently killed trees
1	*Pseudotsuga taxifolia*	Pitted laminated rot in 100-year-old tree
2	*Pseudotsuga taxifolia*	Pitted laminated rot in 200-year-old tree
1	*Pseudotsuga taxifolia*	Rot in root of young living tree with associated purplish-brown mycelial felts
1	*Pseudotsuga taxifolia*	Rot in root of young tree recently killed, with associated purplish-brown mycelium
1	*Pseudotsuga taxifolia*	Context of sporophore on stump of tree fallen two to three years previously
1	*Tsuga heterophylla*	Rot in crown
1	*Tsuga heterophylla*	Rot in stump of freshly cut tree

In addition, the fungus is tested on media containing gallic and tannic acid, following the method described by Davidson, Campbell, and Blaisdell (2). Microscopic examinations are made at suitable intervals, records being kept of the characteristics of the hyphae, occurrence of secondary spores, special structures, etc. Descriptions of the cultures of the *Pseudotsuga* root-rot organism and of *Poria Weirii* follow.

Poria Weirii from Pseudotsuga taxifolia

A. *Macroscopic Characters*

1-week-old cultures.—(Plate IV, Figs. 3, 6) 2.7–3.6 cm. diameter; white; margin sharply defined, of coarse fibres; mat slightly raised, cottony to floccose-cottony, loosely arranged, sometimes with minute tufts scattered over the surface, in some isolates becoming slightly flattened around inoculum; reverse unchanged, odour none. *2-weeks-old cultures.*— (Plate IV, Figs. 4, 7) 6.0–8.4 cm. diameter; white to tilleul-buff, vinaceous-buff, avellaneous and wood brown or cream-buff and chamois, the colour being pale and evenly distributed or limited to certain zones, either near the inoculum or at some distance from it; margin sharply defined; mat slightly raised, cottony in newest growth, becoming woolly, amorphous (i.e., individual fibres no longer evident), and, in some isolates, flattened around the inoculum; reverse unchanged; odour lacking in some isolates, strong and unpleasant in others. *4-weeks-old cultures.*— (Plate IV, Figs. 5, 8). Colours as described above with some isolates developing the deeper tones of honey yellow and tawny-olive; mat slightly raised, uniformly woolly. Two isolates produced small areas crust-like in texture and tawny-olive to Saccardo's umber in colour

(Plate IV, Fig. 8). No fruiting occurred on any of the isolates up to the end of six weeks; reverse unchanged; odour no longer perceptible in any isolates.

Gallic and tannic acid media.—Strong diffusion zones are produced on both these media, with mats 24–45 mm. diameter after 7 days. Hence the fungus goes into Group 7 of Davidson, Campbell, and Blaisdell's scheme (2).

B. *Microscopic Characters*

Advancing zone.—Hyphae (Fig. 12) hyaline, thin-walled, contents staining in phloxine, with simple septa, branched, the branching frequently occurring immediately below a septum, 2.2–6.0 μ diameter. *Aerial mycelium.*—*(a)* hyphae as in advancing zone; *(b)* hyphae (Fig. 13) yellow to brown in potassium hydroxide solution, walls slightly thickened, frequently septate, branched, 2.2–4.5 μ diameter, usually ending in setal hyphae; *(c)* setal hyphae numerous in all parts of mycelial mat, slender, tapering to a point, with walls thick and dark brown in potassium hydroxide, 4.5–6.0 (–7.5) μ diameter, up to 350 μ long (Fig. 14; Plate IV, Fig. 2). *Submerged mycelium.*—*(a)* hyphae as in advancing zone; *(b)* crystals numerous, octahedral.

Poria Weirii from Thuja

A. *Macroscopic Characters*

1-week-old cultures.— (Plate IV, Fig. 9) 2.3–3.2 cm. diameter; white; margin sharply defined; slightly raised, cottony, loosely arranged, dotted with coarser fibres; reverse unchanged; odour none. *2-weeks-old cultures.*— (Plate IV, Fig. 10) 5.8–6.9 cm. diameter; white to cream-buff and chamois; margin sharply defined; outer part of mat raised, cottony, with radiating growth lines; inner part (near inoculum) flattened, appearing thinner, with farinaceous surface; reverse unchanged; odour strong and unpleasant. *4-weeks-old cultures.*— (Plate IV, Fig. 11) colours as described above, deepening to honey yellow and buckthorn brown, the colour being darkest in new growth, paler around inoculum; mat slightly raised in newest parts, cottony to plumose, becoming appressed, farinaceous around inoculum; reverse bleached; odour no longer perceptible.

Gallic and tannic acid media.—Strong diffusion zones with mats 20–40 mm. in diameter are produced on both media; hence the fungus goes into Group 7 of Davidson, Campbell, and Blaisdell's scheme (2) which is in accord with their observations.

B. *Microscopic Characters*

Advancing zone.—Hyphae hyaline, thin-walled, contents staining in phloxine, with simple septa, branched, 1.5–4.5 μ diameter. *Aerial mycelium.*— *(a)* hyphae as in advancing zone; *(b)* hyphae with slightly thickened walls, buff to brown in potassium hydroxide, with numerous conspicuous cross walls, branched, 3.0–6.0 μ diameter; *(c)* setal hyphae numerous in one isolate, but confined to certain isolated patches in the other, slender, tapering to a point, with walls thick and dark brown in potassium hydroxide, 3.0–5.0 μ diameter.

Submerged mycelium.—*(a)* hyphae as in advancing zone; *(b)* crystals numerous, octahedral.

A comparison of cultures of the root-rot organism and of *Poria Weirii* shows that they are similar in rates of growth, character of margin, general appearance of mycelial mat, production of minute dark brown resinous masses against the glass in old cultures, either in Petri dishes or culture tubes, reactions on media containing gallic and tannic acid, and in their microscopic characters. Cultures of *Poria Weirii* from *Thuja plicata* differ from those of the root-rot organism in their colour, which is a deeper yellow to brown rather than the paler avellaneous tones present in most isolates of the latter. This difference in colour is related, apparently, to the greater number of yellow-brown hyphae present in the *Poria Weirii* isolates. On the other hand, setal hyphae are less abundant in *Poria Weirii*, being sparsely distributed in most preparations, whereas in the root-rot organism they are abundant and conspicuous in every mount (Plate IV, Fig. 2) and are noticeable when cultures are examined with a hand lens. Identical structures occur in the isolates of the root-rot fungus and *Poria Weirii*, and the differences in the relative distribution of these structures account for the variations observed. It should be mentioned that the description of *Poria Weirii* is based on only two isolates, and that a greater range of variation might have been encountered had more cultures been available for study.

In spite of the differences recorded above, the comparison of cultures of the root-rot organism from *Pseudotsuga taxifolia* and *Tsuga heterophylla* with those of *Poria Weirii* from *Thuja plicata* has led to the conclusion that the root-rot organism is *Poria Weirii* or a form of it, thereby corroborating the identification based on a study of the decay and sporophores.

Armillaria Root-Rot in *Pseudotsuga*

This disease has caused appreciable loss in young Douglas fir at the Cowichan Lake Forest Experiment Station. Infected trees were distributed over the area on both good and poor sites but whether any correlation exists between the site and the intensity of the disease is not known. *A. mellea* was also found as far north as Campbell River on Vancouver Island and in the Fraser Valley on the mainland. Observations made to date suggest that it may be more prevalent in the Fraser Valley and north of Nanaimo on Vancouver Island than in the Cowichan Lake region.

In young Douglas fir stands, root-rots caused by both *Armillaria mellea* and *Poria Weirii* may be responsible for the stunting of leaves and twigs, discoloration of the crown, and the death of trees either singly, or in groups that are roughly circular in outline. The causal organism is readily determined in the presence of sporophores or of the advanced stage of the decay. Clumps of the honey-coloured mushroom, *A. mellea*, may be found at the base of an infected tree, or the resupinate brown, pored fruit-body of *P. Weirii* on the crown or trunk. The advanced decay caused by *A. mellea* in which "the wood becomes light yellow or white in colour, soft and spongy, often

stringy in conifers, and marked by numerous black zone lines" bears little resemblance to the pitted, laminated rot of *P. Weirii*. In the absence of sporophores or advanced decay *A. mellea* may be recognized because:

(a) it turns the needles pale yellowish to yellowish-brown colour instead of reddish-brown,

(b) an abnormal resin flow is commonly found at the basal region of infected trees. "On occasion this flow is so great that the litter surrounding the root collar becomes compacted into a hard crust,"

(c) veined white mycelial fans are abundant between the bark and wood of infected trees and "constitute one of the most reliable signs of the disease,"

(d) dark brown or black rhizomorphic strands may be present in the cambium, on the surface of diseased roots, and free in the soil,

(e) in the early stages of decay or in case of doubt cultures of the organism are readily obtained and are definitely diagnostic. Those of *A. mellea* are slow growing, develop branched, white rhizomorphs in the agar and a purplish-brown resinous surface growth which would never be confused with the yellow and buff cottony mycelial mat of *P. Weirii*.

Hence, although either of these root-rots of Douglas fir may cause a reduction of the current season's growth, discoloration of the foliage, and the death of the tree they may be distinguished by their sporophores, by the presence or absence of an abnormal resin flow, mycelial fans beneath the bark, and rhizomorphs, by the type of advanced decay, and by the character of the mycelium produced in culture.

Acknowledgments

The authors wish to express their indebtedness to Mr. E. C. Manning, Chief Forester, and to the members of the Research Division of the British Columbia Forest Branch for their interest and generous assistance, and to Dr. H. T. Güssow, Dominion Botanist, for the opportunity to work upon this problem.

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CONTRIBUTIONS TO A STUDY OF THE FUNGUS FLORA OF NOVA SCOTIA

V. DISCOMYCETES[1]

By Lewis E. Wehmeyer[2]

Introduction

The species of Discomycetes presented in this list undoubtedly represent only a small fraction of the flora of the region. Only one summer, that of 1931, was sufficiently moist to yield a representative crop of fleshy forms. During that season a large representation of the Geoglossaceae were found in abundance, whereas only a few scattered collections were obtained in the other, drier, summers. Most of the species of the Helvellaceae and Pezizaceae were also collected during this same summer. The collections made in the remaining seasons were of a scattered nature, obtained in the course of general collecting.

The present arrangement of the Discomycetes is in such an unsettled and incomplete state that no attempt has been made to follow the latest arrangements and taxonomy. The nomenclature followed, in general, is that of Rehm (10).

The localities in which the majority of the collections were made have been described in the first paper of this series (14) and are referred to here merely by name.

The writer wishes to take this opportunity to thank the specialists in various groups who have so kindly aided him in the determination of many of these collections.

DISCOMYCETES

Exoascaceae

Taphrina caerulescens (Mont. & Desm.) Tul. On leaves of *Quercus* sp. Pictou Beach, Pictou Co., July 22, 1931, (1113).

— **communis** (Sadeb.) Gies. On fruits of *Prunus americana*, Bedford, Halifax Co., July 2, 1929, leg. A. R. Prince, (15).

— **Robinsoniana** Gies. On female catkins of *Alnus incana*, Salmon River, July 14, 1931, (1110). Asci from this material were somewhat larger (31–53 × 8.8–10.6 μ) than the measurements given by Ray (9, p. 58), but they are apparently this species.

[1] *Manuscript received March 7, 1940.*

Contribution from the Department of Botany, University of Michigan, Ann Arbor, Michigan. Papers from the Department of Botany, University of Michigan, No. 715.

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Elaphomycetaceae

Elaphomyces muricatus Fr. In hole in tree, New Glasgow Rd., July 25, 1931, (1135); surface of ground, Mt. Thom, Aug. 10, 1931, (1135a), both det. by C. W. Dodge. No. 1135 was gnawed by squirrels and no doubt both specimens were carried by squirrels or other rodents.

Operculatae

Helvellaceae

Gyromitra esculenta Fr. On soil, Glenholme, Colchester Co., June 10, 1925, leg. A. R. Prince, (1098); Folleigh Lake, Oct., 1928, leg. A. R. Prince, (236). Seaver (13, p. 252) considers this as merely a gyrose form of *Elvela infula* Schaeff., which may well be true, but these collections are so distinct in form from No. 1416 that they are kept separate here.

—**(Helvella) infula** (Schaeff.) Quél. On duff under conifers, Upper Brookside, Aug. 27, 1931, leg. A. H. Smith, (1416). Stems 7–10 cm. in length, pileus typically saddle-shaped.

Helvella crispa (Scop.) Fr. On old stump, Earltown Rd., Aug. 22, 1931, (1387).

— **elastica** Bull. On soil, mixed woods, Earltown Rd., Aug. 21, 1931, (1378).

— **lacunosa** Afzel. (*H. Mitra* L.). Among moss, Upper Brookside, July 4, 1931, (381). Mixed woods, Earltown Rd., Aug. 21, 1931, (381a). The stalk of 381a dried a yellowish colour. All gradations of stipe colour seem to occur.

Macropodia macropus Fckl. On rotten logs and moss, Upper Brookside, July 17, 1931, leg. A. H. Smith, (1075); Lake O'Law, Inverness Co., Aug. 6, 1931, (1075a); Mt. Thom, Aug. 10, 1931, leg. A. H. Smith, (1075b). The large central oil globule in the spores of this and the following species of *Macropodia* would place them in the genus *Helvella* as circumscribed by Nannfeldt (6).

— **platypodia** Dodge. On soil, Upper Brookside, July 18, 1931, leg. A. H. Smith, (1080) det. F. J. Seaver.

— **subclavipes** (Phill. & Ell.) Rehm. On moist soil, Victoria Park, (240).

Pezizaceae

Ascophanus carneus (Pers.) Boud. On dung (bear ?), Upper Brookside, July 24, 1931, (1498). Obtained in gross culture. The apothecia have the colour and mycelial strands of *A. testaceus*, but the smaller fruit bodies and rough spores of *A. carneus*. The tips of the paraphyses are broader (4.5–5.5 μ below; 10–14 μ above) than given for *A. carneus*.

— **granulatus** (Bull.) Speg. forma **cervorum** Rehm. On cow dung, Salmon River, Aug. 1, 1931, (1204). This collection is intermediate between *A.* (*Humaria*) *granulatus* and *A. granuliformis* (Crouan) Boud. It has the long asci (80–140 $\times$ 17–23 μ), larger apothecia, and yellowish granular paraphyses of the former, but the smaller spores of the latter. The forma *cervorum*, with many spores 10 μ long, mentioned by Rehm (10, p. 943), seems to be the closest named form.

— **lacteus** (Cke. & Phill.) Phill. On cow dung, Upper Brookside, July 3, 1931, (377). The white, or yellowish-white, species of Ascophanus, *A. argenteus*, *A. lacteus*, *A. microsporus*, and *A. ochraceus* are very much confused and no two authors seem to agree on all characters. This collection agrees best with Rehm's description of *A. lacteus* (10, p. 1086).

Geopyxis catinus (Holmsk.) Sacc. On soil under spruce, Upper Brookside, Aug. 20, 1931, leg. A. H. Smith, (1373).

Humaria xanthomela Cke. var. **americana** (Rehm) Sacc. & Trott. On mossy soil at base of beech, Upper Brookside, Sept. 3, 1931, (1460), det. E. K. Cash.

Lamprospora polytrichina (Rehm) Seaver. On soil, and among mosses, Valley, Colchester Co., July 1, 1929, (8); Salmon River, July 3, 1931, (367). The spores of these collections (11.5–14 μ in diameter) are larger than those given by Rehm (9–10 μ) but agree with the measurements of Cooke, Phillips, and Seaver.

Lasiobolus equinus (Müll.) Karst. On rabbit dung, Salmon River, July 3, 1931, (362).

Otidea leporina (Batsch) Fckl. Decayed wood, Salmon River, Aug. 18, 1931, leg. A. H. Smith, (1344); on soil, Upper Brookside, Aug. 20, 1931, leg. A. H. Smith, (1344a); on humus, Earltown Rd., Aug. 21, 1931, leg. A. H. Smith, (1344b).

Patella (Lachnea) albospadicea (Grev.) Seaver. On bare soil, Salmon River, July 3, 1931, leg. A. H. Smith, (365); North River Rd., Colchester Co., July 7, 1931, (418); Upper Brookside, July 10, 1931, leg. A. H. Smith, (418a), (418b); Upper Brookside, July 27, 1931, (418c). The spores of these collections, in general, run longer (21–24 × 8–11 μ), and in 418b broader (14 μ), than given by Seaver (13, p. 179). The apothecia are often covered by a white cobwebby mycelium.

Lachnea alpina (Fckl.) Sacc. On moose dung, Mt. Thom, Aug. 10, 1931, (1254); on porcupine dung, Mt. Thom, Aug. 10, 1931, (1254a). This is similar to, and has the stellate hairs of *L. stercorea*, but the asci (150–200 × 9–10 μ), spores (12–14 × 7–8 μ), and apothecia (0.5–2 mm.) are definitely smaller. It approaches *Humaria alpina* Fckl. as described by Cooke (1, p. 81), but Fuckel (3, Nachtr. 3 : 32) gives the spores of this species as 16 × 9 μ and says it differs from *L. stercorea* in its colour and the stellate hairs. If *L. alpina* does differ in the smaller spores and apothecia, this is it.

— **coprinaria** (Cke.) Sacc. On cow dung, North River Rd., Colchester Co., July 7, 1931, leg. A. H. Smith, (419); Upper Brookside, Sept. 7, 1931, (419b); on bear dung, Mt. Thom, Aug. 10, 1931, (419a).

— **erinaceus** (Schw.) Sacc. On wet sticks, Jollytown Rd., Colchester Co., July 26, 1929, (79).

According to Seaver, this species differs from *Patella albospadicea* only in its occurrence on wood. The spores of this collection are slightly smaller than those placed in *P. albospadicea*, but fit better Seaver's measurements for that species.

— **hemisphaerica** (Wigg.) Gill. On moss, Lake O'Law, Inverness Co., Aug. 6, 1931, (1220); Wentworth Valley, Cumberland Co., Aug. 29, 1931, (1220a).

— **scutellata** (L.) Gill. On decayed wood, Salmon River, July 3, 1931, (366); Folleigh Lake, July 20, 1931, (366a).

— **setosa** (Nees) Phill. On *Betula*, Upper Brookside, July 4, 1931, (387).

— **stercorea** (Pers.) Gill. On cow dung, Upper Brookside, July 3, 1931, (376). This collection has the stellate hairs of the var. *gemella* Karst. Rehm (10, p. 1057) gives *Humaria alpina* Fckl. as a synonym of this variety. The collections placed under *L. alpina* in this paper have smaller spores. The spores of this collection are 17–19 × 8.5–9.5 μ.

— **umbrorum** (Fr.) Gill. On bare soil, St. Peters, Richmond Co., Aug. 3, 1931, (1237). There is little to choose from between the descriptions of *L. hirta*, *L. scutellata* and *L. umbrorum*. This collection is placed in the latter species on account of the larger roughened spores, the shorter, sparser hairs and the occurrence on soil. The spores of this collection measure 21–24.5 × 11–12 μ.

Peziza ampelina Quél. On rocky bank, Upper Brookside, July 4, 1931, (389). These plants have the curved violet-brown paraphyses of *P. viridaria* and *P. violacea* and the small apothecia (5–12 mm.) of the former. The spores (14–17 × 7.5–9.5 μ) are, however, larger than those given for either of these two species. They come nearest to *P. ampelina*, although the apothecia are smaller and lighter in colour than given for that species.

— **atrovinosa** Cke. & Ger. On soil, Salmon River, Aug. 18, 1931, leg. A. H. Smith, (1348). This collection is characterized by the small apothecia, 1.5–4 cm. in diameter, which become expanded but not repand. They have a dark brown hymenium which becomes intense black at maturity. The asci are cylindric, 200–250 × 11–12 μ and turn blue with potassium iodide. The spores are broad-ellipsoid, coarsely tuberculate-ridged, hyaline and 12.5–14 × 8.5–9 μ. The paraphyses are terete, simple or slightly branched, 3.5 μ in diameter and expanded above (6–7 μ). It is related to *P. badia* but has smaller apothecia with a very dark hymenium and has smaller spores. It seems to fit best the above species. It is also near *P. chlamydospora* E. & E.

— **badia** Pers. On soil, Upper Brookside, July 26, 1929, (132); July 27, 1929, (174); July 18, 1931, leg. A. H. Smith, (295b); Sept. 3, 1931, leg. A. H. Smith, (1030b). On moss-covered wood, Victoria Park, June 24, 1931, (295).

The only apparent difference between *P. badia* and *P. brunneo-atra* is the larger apothecia of the former. The Nova Scotian plants placed in these two species showed all intergrading apothecial sizes, but they all dried a dark black and had hyaline to pale yellow-brown, roughened spores measuring 15–19 × (7)8–10 μ. The paraphyses were septate and yellow-brown except in Nos. 1030a and 1030b, where they were sparse, hyaline, and non-septate. Nos. 132 and 295b showed the olive colour of the hymenium given for *P. cervina* Fckl. The plants placed under *P. badia* include those with larger apothecia, 2.5–4 cm. in diameter.

— **brunneo-atra** Desm. On soil, Salmon River, July 15, 1931, leg. A. H. Smith, (295a); Victoria Park, July 23, 1931, (1030a). On sawdust, Salmon River, July 14, 1931, (1030). The collections placed here had apothecia 12–25 mm. in diameter.

— **fimeti** (Fckl.) Seaver. On cow dung, Upper Brookside, July 7, 1931, (378).

The spores of these plants (17.5 –19 × 8.5–9.5 μ) are larger than the measurements of Seaver (8 × 16 μ) but agree with those of Rehm (15–18 × 9–10 μ).

— **Howsei** Roze & Boud. On soil, Upper Brookside, Aug. 16–20, 1931, leg. A. H. Smith, (1343, 1343b, 1343c); Sept. 3, 1931, (1343d). On decayed log, Salmon River, Aug. 18, 1931, (1343a). The collections placed here have been difficult to determine. In general, the presence of violet shades in the hymenium or, at least in the hypothecium, suggests *P. violacea*. They do not have the violet, curved paraphyses of that species, and the spores are definitely roughened at maturity, however. Seaver (13, p. 226) gives the spores of *P. violacea* as "sculptured," but these collections have spores definitely larger than *P. violacea* (10–14 × 5–9 μ, after various authors). These plants have a white punctate exterior and dry translucent or furfuraceous yellow-brown, with the appearance of a dried peach. They seem to fit best Roze and Boudier's *P. Howsei* (11). An inclusive description follows.

Apothecia 0.8–5 cm. in diameter, at first globose then cup-shaped to repand or undulate, often with a short stipe-like base. Young plants a clear pale lavender, becoming darker, eventually dull yellow-brown or olivaceous due to the browning of the hymenium. Lavender tints usually present in the hypothecium. Exterior white, punctate, furfuraceous. Asci cylindric, 250–300 × 14–17 μ, blue with potassium iodide. Spores hyaline, ellipsoid, biguttulate, somewhat roughened at maturity, 14–19 × 7–10 μ. Paraphyses sparse, simple, filiform with slightly enlarged tips, 3.5–4.5 μ in diameter.

The purple tints were mostly lacking in 295c, but the appearance of the dried plants was similar to all of the other collections, which is quite distinct from the dried plants of *P. badia*.

— **pustulata** (Hedw.) Pers. On soil, Upper Brookside, Aug. 18, 1931, leg. A. H. Smith, (1351). Apparently immature; paraphyses hyaline and not thickened at the apex. Hymenium brown with a violet cast.

— **repanda** Pers. On decayed log, Lake O'Law, Inverness Co., Aug. 6, 1931, (1223).

— **sepiatra** Cke.? On decayed sticks, Upper Brookside, July 10, 1931, leg. A. H. Smith, (481). Apothecia 1–2 cm. in diameter, soon repand, tough, externally dirty white with an olivaceous cast. Hymenium brown, smooth. Asci cylindric, 250–300 × 14–16 μ, blue with potassium iodide. Spores ellipsoid, hyaline, smooth, biguttulate, 19–22 × 10–13 μ. Paraphyses simple, apex clavately enlarged, 5–7 μ, somewhat curved or bent; tips filled with yellow-brown granules. This collection does not fit the description of any species exactly. It is lighter in colour than *P. sepiatra* and *P. clypeata*, has smaller spores than the latter, and has larger spores than *P. subrepanda*.

— **succosa** Berk. On bare soil, Victoria Park, Aug. 16, 1929, (242); Upper Brookside, July 10, 1931, (480).

— **sylvestris** (Boud.) Sacc. & Trott. On soil, Victoria Park, June 24, 1931, (294). This collection differs from No. 329 of *P. vesciculosa* only in the smaller apothecia (1–2 cm. in diameter) with less well developed stipitate base and in the smaller spores (17.5–19 × 10 μ). Rehm (10, p. 1018) places such material in *P. vesciculosa* var. *cerea* (Sow.) Rehm, but Seaver places them under *P. sylvestris*. This differs from *P. fimeti* only in the slightly lighter hymenium and the habitat.

Peziza vesciculosa Bull. On bare soil, Upper Brookside, June 29, 1931, leg. A. H. Smith, (329).

Typical of *P. vesciculosa*, but not giving a blue iodine test. Spores larger (19–23 × 10–11 μ) than in *P. sylvestris*.

Saccobolus Kerverni (Crouan) Boud. On dung (bear ?), Upper Brookside, July 24, 1931, (1497). Apothecia (200–400 μ) and spores (17–21 × 6.5–8.5 μ) somewhat smaller than given in descriptions.

Sphaerospora brunnea (Alb. & Schw.) Massee. Among moss on charred logs, Victoria Park, July 16, 1931, (406).

Inoperculatae

Geoglossaceae

Apostemidium fiscellum Karst. On submerged wood, Salmon River, Aug. 19, 1933, (1638).

Apothecia yellow-brown when fresh, turbinate, 1–2 mm. diameter, drying black with a crenulate margin. Excipulum of pale brown parenchyma with a dark brown cortex, 30–120 μ thick, thicker at the base than at the margin. Asci filiform, cylindric, 120–150 × 5–6 μ. Spores parallel, filiform, hyaline, 85–100 × 1 μ. Paraphyses filiform, simple, clavate thickened at the apex, 1.5 μ diam., hyaline.

The shorter asci and spores are the characters used to distinguish *A. fiscellum*, which is an European species not reported from America by Durand (2, p. 457).

Corynetes robustus Durand. On soil under hardwoods, Upper Brookside, Aug. 27, 1931, leg. A. H. Smith, (1426); Folleigh Lake, Aug. 29, 1931, (1426a). Both of these collections fit very well Durand's description except for the fact that the ascigerous portion is sharply delimited from the stem in these plants.

Cudonia circinans (Pers.) Fr. Among moss or on duff, usually under spruce or other conifers, Middle River, Victoria Co., Aug. 5, 1931, (1216) (1216a); Salmon River, Aug. 18, 1931 and Sept. 2, 1931, (1216d) (1216f); Upper Brookside, Aug. 12, 1931, (1216b), Aug. 17 and 20, 1931, leg. A. H. Smith, (1216c) (1216e). All of these collections showed numerous budding ascospores, to such an extent that many asci were completely filled with the small ellipsoid secondary conidia which are one-celled and 3.5–6 × 1.5–2 μ. The primary ascospores were long fusoid to clavate, 31–49 × 1.5–3 μ. Although not given in his description of *C. circinans*, these budding secondary conidia are mentioned elsewhere by Durand (2, p. 388).

— **lutea** (Pk.) Sacc. On leaves of *Fagus*, Upper Brookside, Aug. 14, 1931, (1304); Victoria Park, Aug. 17, 1931, leg. A. H. Smith, (1304a).

Geoglossum fallax Durand. Among moss under hardwoods, Lake O'Law, Inverness Co., Aug. 6, 1931, (1219).

— **glabrum** Pers. On soil, humus or much decayed wood, Westcook's Cove, Guysborough Co., leg. A. R. Prince, Sept. 5, 1925, (1159); Princeport, Colchester Co., Aug. 26, 1927, leg. A. R. Prince, (6111); Mt. Thom, Pictou Co., Aug. 11, 1927, leg. A. R. Prince, (6109); Victoria Park, July 23, 1931, (1130); Upper Brookside, July 24, 1931, (1130a); Folleigh Lake, Aug. 19, 1931, (1130b); Earltown Rd., Aug. 19, 1931, (1130c).

— **nigritum** Cke. On soil, Folleigh Lake, Aug. 18, 1927, leg. A. R. Prince, (6099); Earltown Rd., Aug. 19, 1931, leg. A. H. Smith, (1363). Collection No. 1363 contains minute plants, 1–2 cm. tall. The paraphyses are those of *G. nigritum* and the spores are 40–55 × 5–6 μ. This seems to be the dwarf, poorly developed form mentioned by Durand (2, p. 427) as being described as *G. glutinosum* var. *minus* Sacc. No. 6099 is somewhat intermediate between this species and *G. glabrum*. The paraphyses are typical of *G. nigritum* when young, but become more darkly coloured, more closely septate and somewhat moniliform in age. The spores measure 75–90 × 7.5 μ.

— **affine** Durand. On mossy soil, Wentworth Valley, Cumberland Co., Aug. 29, 1931, leg. A. H. Smith, (1436).

— **difforme** (Fr.) Durand. On decayed stump, Earltown Rd., Aug. 22, 1931, (1386); on duff and humus under hardwoods, Upper Brookside, Aug. 24, 1931, (1386a); Aug. 27, 1931, leg. A. H. Smith, (1386b); Wentworth Valley, Cumberland Co., Aug. 29, 1931, leg. A. H. Smith, (1386c).

— **glutinosum** (Pers.) Durand. On humus, Earltown Rd., Aug. 21, 1931, leg. A. H. Smith, (1381).

Leotia lubrica (Scop.) Pers. On wet soil, Princeport, Colchester Co., Aug. 26, 1927, leg. A. R. Prince, (6032). On conifer duff, Upper Brookside, Aug. 14, 1931, (440a).

— **lubrica** forma **Lloydii** (Rehm) Durand. Among moss under conifers, Victoria Park, Sept. 10, 1927, (440). Typical of Durand's (2, p. 447) description.

— **stipitata** (Bosc.) Schroet. Among moss on duff, Victoria Park, Sept. 10, 1927, (441); Salmon River, Aug. 18, 1931, leg. A. H. Smith, (441a).

Microglossum rufum (Schw.) Underw. On soil and duff, Upper Brookside, Aug. 26, 1927, leg. A. R. Prince, (6217); Economy River, Colchester Co., Aug. 31, 1927, leg. A. R. Prince (6112); Northeast Margaree, Inverness Co., Sept. 4, 1927, (439); Folleigh Lake, July 20, 1931, (1099); Earltown Rd., Aug. 19, 1931, (1099a).

— **viride** (Pers.) Gill. On soil, mixed woods, Wentworth Valley, Cumberland Co., Aug. 29, 1931, leg. A. H. Smith, (1432). These plants have the smooth fibrous stem of *M. olivaceum* but the spores are those of *M. viride* (17–23 × 5–6 μ).

Spathularia clavata (Schaeff.) Sacc. On soil or duff, Princeport, Colchester Co., Aug. 26, 1927, leg. A. R. Prince, (6222); Upper Brookside, Aug. 14, 1931, (63a); Sept. 9, 1931, leg. A. H. Smith, (63b).

— **velutipes** Cke. & Farl. On decayed wood and humus, Upper Brookside, Aug. 1927, leg. A. R. Prince, (6216); Killag Mines, July 30, 1931, (52a).

Trichoglossum Farlowii (Cke.) Durand. On soil under hardwoods, Wentworth Valley, Cumberland Co., Aug. 29, 1931, leg. A. H. Smith, (1435).

— **hirsutum** (Pers.) Boud. On soil, sandy creek, Wentworth Valley, Cumberland Co., Aug. 29, 1931, leg. A. H. Smith, (1437).

— **velutipes** (Pk.) Durand. On soil, mixed woods, Earltown Rd., Aug. 21, 1931, (1380).

Vibrissea truncorum (Alb. & Schw.) Fr. On submerged sticks in cold stream, Halifax Co., June 27, 1926, (P376).

Phacidiaceae

Coccomyces Cembrae Rehm. On stems of *Larix laricina*, Upper Brookside, July 13, 1931, (1004).

— **coronatus** (Schum.) Rehm. On dead leaves of *Fagus*, Upper Brookside, Sept. 3, 1921, (1467).

— **coronatus** var. **trigonus** (Schm. & Kze.) Karst. On dead leaves of *Fagus* and *Acer*, Upper Brookside, Aug. 11, 1931, (1277). On dead leaves of *Populus tremuloides*, Salmon River, Aug. 1, 1931, (1198). These collections differed from No. 1467 in the presence of definite rounded brown spots, definitely margined by a blackened zone, the fusoid character of the apothecia on these spots and the smaller spores (25–37 × 1–3 μ). The apothecia also have a raised ridge-like margin. A wide variation of form is found in exsiccati of *C. coronatus*, however, and apothecia of this same appearance are found in Syd., Myc. Germ. 1611, for instance. This is probably the var. *trigonus*, with shorter spores.

Coccophacidium Pini (Fr.) Rehm. On stems of *Pinus Strobus*, Victoria Park, Aug. 30, 1933, (1647).

Rhytisma Ilicis-canadensis Schw. On leaves of *Nemopanthus mucronata*, Great Anse, Richmond Co., Aug. 3, 1931, (1234); Upper Brookside, July 28, 1931, (1234a).

Stictidaceae

Ocellaria ocellata (Fr.) Schroet. On stems of *Populus* and *Salix* spp., Upper Brookside, July 29, 1929, (150); Aug. 2, 1929, (196); June 27, 1931, (303); Victoria Park, June 26, 1935, (1710).

The spores of these collections appear to run larger than measurements given for European collections. Most European citations run from 21–30 × 9–12 μ. Fuckel (3, Nachtr. 1 : 326) gives them as 40 × 12 μ. Wollenweber (15, p. 569) cites the spores in 8-spored asci as 23–28 × 9–12 μ, occasionally 20–40 × 8–14 μ, and those in 4–6-spored asci as 25–40 × 8–14 μ. The spores of these collections were (21)26–43 × (11.5)13–16 μ.

Propolis faginea (Schrad.) Karst. On bark and wood of *Fagus*, Upper Brookside, July 14, 1929, (57) June 28, 1931, (316); July 16, 1931, (1066); June 19, 1933, (1665); Victoria Park, Aug. 8, 1929, (218); Green Oaks, Colchester Co., July 9, 1931, (470).

Common, asci 90–106 × 12–15 μ, spores 20–26.5 × 5.5–7 μ.

— **Leonis** (Tul.) Rehm. On twigs and cone scales of *Picea*, Upper Brookside, July 4, 1931, (485); July 16, 1931, (1063); Salmon River, Sept. 7, 1931, (1479). These collections on *Picea* differ definitely from *P faginea* in the asci (130–200 × 20–23 μ), which in *P. Leonis* are more cylindric-clavate and long stalked and become more elongate with the spores arranged uniseriately, and the spores (23–31 × 9–14 μ), which are longer, have a thicker outer wall (2–2.5 μ), a granular rather than a biguttulate content, and are less strongly curved. *P. Leonis* is given as having a greenish hymenium, but Ell., N.A.F. 73, which was compared with *Stictis Leonis* Tul., and found to be the same, has a whitish hymenium as do Rel. Farl. 140 and Fl. Fayette Co., 1581 of *S. Leonis*. *Propolis Hillmanniana* W. Kirschst. (5, p. 203) may be this same species, although Kirschstein gives the spores of his species as being 40–50 × 10–14 μ.

Stictis radiata (L.) Pers. On bark of *Acer spicatum* and *A. saccharum*, Upper Brookside, July 15, 1929, (66); Salmon River, July 15, 1931, (1044). On decorticated *Betula*, Salmon River, July 15, 1931, (1036). These collections on bark differ from *S. mollis* in the snow white rather than greyish lips and the light coloured hymenium. The spores measure 190–250 × 1.5–2 μ.

Patellariaceae

Calicium abietinum Pers. On coniferous wood, Upper Brookside, July 11, 1931, det. J. H. Lowe, (490); July 22, 1929, (106).

Durella compressa (Pers.) Tul. On decorticated hardwood, Upper Brookside, June 29, 1931, (321), det. E. T. Butler.

— **lecideola** (Fr.) Rehm. On coniferous wood, Portapique Beach, Colchester Co., Aug. 7, 1933, (1618), det. E. T. Butler.

Hysteropatella minor (Cke.) Rehm. On decorticated *Betula*, Salmon River, July 15, 1931, (1077), det. E. T. Butler; North River Rd., July 7, 1931, (443). On decorticated Fagus, Upper Brookside, Aug. 11, 1931, (1662).

Karschia lignyota (Fr.) Sacc. On *Peniophora*, Upper Brookside, Aug. 3, 1929, (212); Salmon River, Aug. 18, 1931, (1355). The spores (7–9 × 3.5 μ) of these collections are somewhat smaller than given by Rehm for this species (9–12 × 4–5 μ).

Mycocalicium pallescens (Nyl.) Vain. On decorticated birch, Folleigh Lake, Aug 29, 1931, (1668), det. J. H. Lowe. Lowe (in litt.) states that this collection "lies between *Mycocalicium albonigrum* (Nyl.) Fink, ... *Chaenothecopsis pusiola* (Ach.) Vain. and *M. pallescens* (Nyl.) Vain. The last two differ essentially in having, or not having, respectively, an associated algal symbiont. I cannot find any algae in your material. Fink's description is too meagre to hazard a guess." Fink's material of *M. albonigrum*, in the Michigan Herbarium, is also meagre, consisting only of a few broken stalks. The Nova Scotia material is consequently referred to *M. pallescens*, which Lowe states is new to North America.

Patinella punctiformis Rehm. On dead wood, Economy Lake, Colchester Co., June 16, 1926, (P378). On decorticated fir, Portapique Beach, July 30, 1933, (1600).

Dermateaceae

Ascocalyx Abietis Naum. On *Abies balsamea*, North River Rd., Colchester Co., July 7, 1931, (416); Upper Brookside, June 19, 1933, (1524), det. J. W. Groves. The apothecia on No. 1524 are accompanied by the curious conidial stage, *Bothrodiscus pinicola* Shear, which has been connected with this species by Groves (4). These collections agree in all respects with Groves' descriptions.

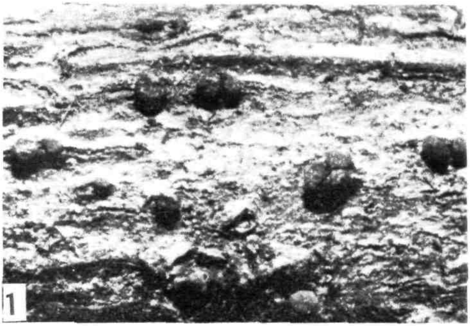
Dermatea Ariae (Pers.) Tul. On *Sorbus americana*, Victoria Park, July 4, 1931, (402). Accompanied by the pycnidial stage, *Micropera Sorbi* (Fr.) Sacc. (*Sphaeronema pallidum* Pk.).

— **balsamea** (Pk.) Seav. On *Abies balsamea*, Upper Brookside, July 8, 1931, (439); July 18, 1931, (1082). On *Tsuga canadensis*, Victoria Park, Aug. 20, 1935, (1791).

All three collections are accompanied by the conidial stage, *Gelatinosporium abietinum* Pk.

— **Cerasi** (Pers.) Fr. On *Prunus* sp., Upper Brookside, Sept. 4, 1931, (1471), det. J. W. Groves. Accompanied by the conidial stage, *Micropera drupacearum* Lév.

— **molliuscula** (Schw.) Cash. On *Betula* sp., Upper Brookside, July 31, 1931, (1194); Aug. 12, 1931, (1287); Aug. 14, 1931, (1287a), det. J. W. Groves. This species fruits abundantly on down branches and cut brush of birch, appearing in the early summer as conidial stromata and in late summer as the dark brown to black apothecia. No. 1287 shows a conidial stage which is probably *Gelatinosporium fulvum* Pk. The elongate yellowish pulverulent stromata are erumpent through the periderm and contain one or many irregular cavities which open to the exterior. The conidiophores lining these cavities bear long, lunate-fusoid, hyaline, one-celled conidia measuring 45–60 × 3–3.5 μ. Collection No. 1194 shows similar stromata with cavities on the surface or more deeply seated. The finer hymenium of these cavities bears smaller conidia which are crescent shaped, hyaline one-celled, tapered at the ends and 7–12 × 1 μ. These conidia apparently represent a *beta* type of conidium for this species.



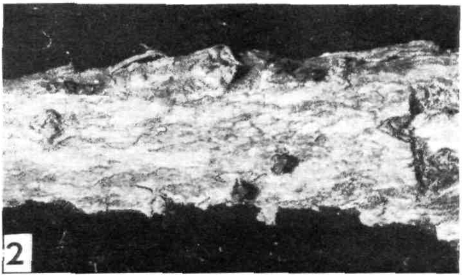


PLATE I, FIGS. 1 – 2. FIG. 1. *Surface view of apothecia of Pezicula Grovesii sp. nov.* ×*12*. FIG. 2. *Surface view of apothecia of Tympanis acericola Groves, sp. nov.* ×*4*.

Godronia turbinata (Schw.) Farl. On *Diervilla Lonicera*, Victoria Park, July 6, 1931, (408); June 21, 1933, (1525); Portapique Beach, Aug. 3, 1933, (1613). Usually to be found on dead stems of this host.

— **urceolus** (Alb. & Schw.) Karst. **var. conferta** Hone. On *Prunus* sp., Salmon River, July 14, 1931, (1020), det. E. K. Cash. Miss Cash, in a letter, states "I have been referring Godronias on several host plants to *G. Urceolus*, as I have not been able to distinguish them from that species morphologically, but it is possible that the forms on different hosts are specifically distinct. . . . . . . This one on Prunus has shorter spores than typical *G. Urceolus* and apothecia usually in dense clusters, although this character is not apparent in your specimen. Both this Nova Scotia collection and one on Prunus from New Hampshire that I have examined, have a *Sirodiplospora* species associated with the *Godronia*, with two-celled fusoid spores borne on branched conidiophores."

Pezicula acericola (Pk.) Sacc. On *Acer saccharum*, Upper Brookside, July 15, 1929, (75); Salmon River, Sept. 7, 1931, (1060a). On *Acer spicatum*, Upper Brookside, July 26, 1929, (82); July 16, 1931, (1060). Common on living or dead bark of standing maple.

— **Alni** (Fck.) Rehm. On *Alnus* sp., Portapique Beach, Colchester Co., July 27, 1933, (1602), fide J. W. Groves.

Pezicula Grovesii sp. nov. (*Figs. 1 and 3–5.*)

Apothecia primum per rimas peridermatis minutas visibilia, discum obscurum vix visibile albidulum formantia, demum erumpentia, parva, diametro 0.2–0.4 mm., solitaria vel bina-quaterna aggregata, flavobrunnea vel pallide olivacea, aetate atriora, convexa, in stipite brevi, hyalino, parenchymatico, stromatico elevata; ascis crassis clavatis, basi angustatis, membrana paululum incrassata, 90–125 μ longis, 18–23 μ crassis; paraphysibus longis, filiformibus, simplicibus, diam. 1 μ, apice abrupte valideque expansis, ellipsoideis, brunneis, 8.5–14.0 μ longis, 5–7 μ latis, ascos superantibus et epithecium formantibus; sporis sursum biseriatis, demum uniseriatis, ellipsoideis vel inaequilateralibus vel paulum curvatis, hyalinis, granuliferis, 1-cellulis, 17–30 μ longis, 8–11 μ crassis. Specimen typicum in *Rhododendro canadensi* loco dicto "Upper Brookside," Nova Scotia, 8 Jul. 1931, in herbario L. E. Wehmeyer, n. 432.

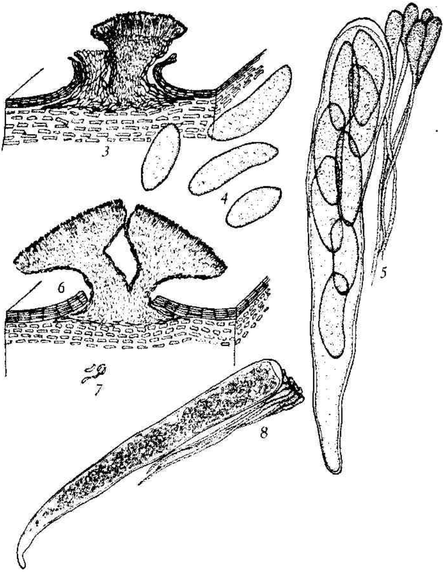
Apothecia (Fig. 1 & 3) preceded by a minute rupture of the periderm which exposes a barely visible whitish disc. Apothecia minute, 0.2–0.4 mm. in diameter, scattered singly or in small groups of two to four, yellowish-brown to pale olive-brown, drying darker, convex, erumpent on a short hyaline, parenchymatic, stromatic stalk, 200–300 μ in diameter. Asci (Fig. 5) stout clavate, with a tapered base and a slightly but evenly thickened wall, 90–125 × 18–23 μ. Paraphyses long filiform, simple, 1 μ in diameter, abruptly and strongly swollen above into ellipsoid, brown tips, 8.5–14 × 5–7 μ, projecting above the asci and forming an epithecium. Spores (Fig. 4) biseriate above, uniseriate below, ellipsoid to inaequilateral or slightly curved, hyaline, granular, one-celled, 17–30 × 8–11 μ. Type collection on *Rhododendron canadense*, Upper Brookside, Nova Scotia, July 8, 1931, in Herbarium L. E. Wehmeyer, No. 432.

The species name is chosen in commemoration of the excellent work done on the genus *Pezicula* by J. W. Groves. Material of this collection was sent to both J. W. Groves and Miss E. K. Cash, neither of whom were able to identify it as any known species. The writer, therefore has taken the responsibility of describing it as new.

The stroma arises on the surface of the bark cortex as a hyaline parenchyma with more or less parallel arranged cells measuring about 12–17.5 × 3.5–7 μ. This stroma ruptures the periderm and expands at the apex, appearing as a whitish disc. The hymenium soon forms over the entire convex surface and the walls of the surface cells of the stroma become darkened, giving the colour to the apothecium.

— **minuta** Pk. On *Viburnum Lentago*, Upper Brookside, July 13, 1931, (1002), det. J. W. Groves.

Sclerroderis Spiraeae Rehm. On *Spiraea tomentosa*, Oldham, Halifax Co., Sept. 5, 1929, (276). On *Spiraea* sp., Upper Brookside, July 28, 1931, (1171), det. E. K. Cash.



FIGS. 3 – 8. FIGS. 3 – 5. *Pezicula Grovesii sp. nov.* FIG. 3. *Vertical section of apothecium.* FIG. 4. *Ascospores.* FIG. 5. *Ascus and paraphyses.* FIGS. 6 – 8. *Tympanis acericola Groves. sp. nov.* FIG. 6. *Vertical section of apothecia.* FIG. 7. *Secondary ascospores.* FIG. 8. *Ascus with secondary ascospores and paraphyses.*

Tympanis acericola Groves sp. nov. (Figs. 2 and 6–8.)

Apothecia erumpentia, dispersa, caespitosa, raro solitaria, subsessilia, brevissime stipitata, orbicularia, vel undulata, 0.5–1.5 mm. lata, 0.5–1.0 mm. alta, nigra, dura, siccitate cornea vel cartilaginea, nondum siccata coriacea; hymenio plano vel convexo, margine primum elevato dein evanescenti; hypothecio plectenchymatico; ascis cylindrico-clavatis, breviter stipitatis, primum octosporis demum multisporis, (75)–85–110–(120) × (10)–12–15 μ; ascosporis primariis late ellipsoideis; 1-2-cellularis, hyalinis, uniseriatis, 4–5 × 2.5–3.5 μ; ascosporis secundariis minutissimis, hyalinis, allantoideis, 1.5–2.5 × 0.5 μ; paraphysibus hyalinis, filiformibus, septatis, furcatis, 1.5–2.5 μ diam., apice incrassatis longitudine usque as 3.4 μ conglutinatis, epithecium fuscum formantibus.

Hab.: in cortice *Aceris spicati* Lam.

Apothecia (Fig. 2 and 6) erumpent, scattered, caespitose in clusters of 2–8, occasionally single, subsessile, very short stipitate, circular, or undulate, 0.5–1.5 mm. in diameter, 0.5–1.0 mm. in height, black, hard, horny to cartilaginous in consistency, becoming tough-leathery when moist, hymenium plane to convex, at first with a raised margin which later disappears; tissue of the hypothecium plectenchymatous, hyaline to yellowish-brown, darker at the outside, forming a rind-like excipulum, the hyphae embedded in a matrix, apparently composed of the thickened, gelatinized walls. Asci (Fig. 8) cylindric-clavate, short stalked, at first eight-spored and very thick-walled, finally many-spored

and with much thinner walls, (75)–85–110–(120) × (10)–12–15 μ. Primary ascospores broadly ellipsoid, one- or two-celled, hyaline, uniseriate, 4.0–5.0 × 2.5–3.5 μ; secondary ascospores (Fig. 7) minute, hyaline, allantoid, 1.5–2.5 × 0.5 μ. Paraphyses hyaline, filiform, septate, much branched toward the tip, 1.5–2.5 μ in diameter, the ends clavate, swollen to 3–4 μ in diameter, and glued together to form a brownish epithecium.

Host: *Acer spicatum* Lam.

Type: Herbarium of J. W. Groves, 516, on *Acer spicatum*, Salmon River, Colchester Co., Nova Scotia, Sept. 7, 1931, leg. L. E. Wehmeyer (1476).

The only other *Tympanis* species heretofore described as occurring on *Acer* is *Tympanis acerina* Rehm on *Acer campestris*. In the description (12, p. 714) no mention is made of the primary ascospores, and the asci are given as slightly longer, 120–130 μ. In the Nova Scotia specimens only an occasional ascus reached 120 μ in length, but in other respects they agreed closely. In the genus *Tympanis*, the asci are apt to vary considerably in length, but the width is usually fairly constant, so that this difference may not be significant. This may be, therefore, the same fungus as that described by Rehm, but no specimens have been available for comparison. In any case, Rehm's name is invalid according to the International Rules, since the combination *Tympanis acerina* has already been used by Peck (7, p. 48) for the fungus now known as *Dermatea acerina* (Pk.) Rehm.

The above description and notes have been submitted by J. W. Groves, who kindly examined this material.

— **alnea** (Pers.) Fr. On *Alnus* spp., Green Oaks, Colchester Co., July 12, 1929, (176); Truro, July 12, 1929, (226); Victoria Park, Sept. 7, 1929, (284); Grande Anse, Richmond Co., Aug. 3, 1931, (1239), fide J. W. Groves. There are two distinct growth forms in these collections. Nos. 226 and 1239 have rather large apothecia, 0.5–1 mm. in diameter, very little incurved margin, are shiny black and occur in small groups of one to three with short stout stalks, often fused almost to the apex. In Nos. 176 and 284, on the other hand, the apothecia occur in large clusters of 5 to 20, are smaller (0.3–0.5 mm.), are definitely cup shaped with inrolled margins, and often show a whitish pulverulence. The stalks are also longer, narrower, and more distinct. The asci and spores appear to be the same in each group. Dr. Groves has kindly examined these collections and states (in litt.) that the pruinose character has been used to separate the species *T. conspersa*, but that he is convinced this is not a specific character. As to the striking differences in apothecial form he is more in doubt. Nos. 226 and 1239 probably represent *Tympanis hysterioides* Rehm. Groves states that the primary ascospores seem to be the same, however, and cultures he has succeeded in obtaining from both types of apothecia are identical.

— **fasciculata** Schw. On *Viburnum Lentago*, Upper Brookside, July 13, 1929, (272), fide J. W. Groves.

— **hysterioides** Rehm. See remarks under *T. alnea*. Nos. 226 and 1239 may be referred to this species.

— **Pinastri** Tul. On *Abies balsamea*, Moore's Lake, Halifax Co., July 6, 1929, (30 and 31); Upper Brookside, Aug. 20, 1931, (1658); Victoria Park, June 25, 1935, (1717), fide J. W. Groves. The collections of this species also show two distinct growth forms. Nos. 30 and 1658 have somewhat larger apothecia (0.6–1 mm.) which are flat-topped with a slightly raised and sometimes serrate margin. These apothecia are borne singly or two to three in a cluster and have short stalks. Nos. 31 and 1717 show larger clusters (3–10) of smaller apothecia (0.5–0.8 mm.) which are somewhat concave with a slight margin. The stalks of these apothecia are stout but more elongate (1–1.2 mm.). The writer was at first inclined to call Nos. 30 and 1658 *T. pithya* (Fr.) Karst. Groves (in litt.) believes, however, that they are merely growth forms and should be called *T. Pinastri*, inasmuch as cultures from both forms show identical colonies. A collection (No. 1659) of the

conidial stage, *Pleurophomella Pinastri* (Penz. & Sacc.) v. Hohn., has also been made on *Abies*.

— **pithya** (Fr.) Karst. See discussion under *T. pinastri*.

— **spermatiospora** Nyl. On *Populus*, Victoria Park, June 27, 1935, (1709).

Orbiliaceae

Orbilia inflatula Karst. On *Fagus grandifolia*, Victoria Park, Aug. 20, 1935, (1666), det. E. K. Cash.

— **leucostigma** Fr. var. **xanthostigma** (Fr.) Rehm. On *Polyporus betulinus*, Upper Brookside, July 30, 1929, (142). This may be *O. coccinella* var. *delicatula*, but it has the rosy colour when fresh and the smaller spores (3–3.5 × 1.5–2 μ) assigned to the above variety by Rehm (10, p. 455).

Orbilia luteorubella (Nyl.) Karst. On decayed stick, Upper Brookside, July, 1929, (45), det. E. K. Cash.

Mollisiaceae

Belonidium pruinosum (Jerd.) Rehm. On stromata of Hypoxylon, on *Fagus*, Victoria Park, June 26, 1935, (1702).

Mollisia benesuada (Tul.) Phill. On *Betula* sp., Victoria Park, Aug. 31, 1929, (266), det. E. K. Cash.

— **caespiticia** Karst. On stromata of *Nectria* and on bark, on *Acer* sp., Upper Brookside, July 17, 1935, (1746). The apothecia occur both on the stromata of the *Nectria* and also on the bark surface. When formed on the stromata, the young apothecia arise completely buried in the nectriaceous stroma.

— **cinerea** (Batsch) Karst. On decayed wood, Upper Brookside, July 14, 1929, (64); July 24, 1929, (97); June 27, 1931, (302); July 1, 1931, (352 and 358); July 7, 1931, (422).

— **melaleuca** (Fr.) Sacc. On decayed wood, North River Rd., Colchester Co., July 7, 1931, leg. A. H. Smith, (427), det. E. K. Cash.

— **stictella** Sacc. & Speg. On *Cornus alternifolia*, Upper Brookside, June 16, 1931, (1056). On *Spiraea* sp., Upper Brookside, July 13, 1931, (498). On *Acer spicatum*, Upper Brookside, July 16, 1931, (1056a). All determined by E. K. Cash.

— **uda** (Pers.) Gill. On blackened wood, Earltown Rd., Aug. 22, 1931, (1392), det. E. K. Cash.

Pseudopeziza Medicaginis (Lib.) Sacc. On alfalfa, East Cheboque, Yarmouth Co., July 30, 1931, leg. N. K. Andrews, det. A. R. Prince.

— **Artemisiae** (Lasch) Rehm. On *Solidago* sp., Truro, June 19, 1933, (1518). Both this species and the following variety are somewhat similar and common on dead *Solidago* stems. The perithecia of this species are smaller and darker at first and do not become so large, although they may become widely erumpent. They are practically sessile, have an exciple of dark brown, thick-walled parenchyma which appears black, and a margin which may be whitish but is not ciliate. The asci (33–38 × 3–4 μ) and spores (5–8.5 × 1–1.5 μ) are also smaller than those of the variety.

Pyrenopeziza Artemisiae var. **Solidaginis** Rehm. On *Solidago*, Upper Brookside, Aug. 1, 1929, (195); Truro, June 19, 1933, (1517). The apothecia of this variety are quite variable. At first they are almost spherical and dark coloured, with a central pore and a darker hymenium. Under favourable moisture conditions, they become widely erumpent and up to 1 mm. in diameter. They are often confluent, pale reddish-brown and have a light coloured hymenium. The stalk is also more pronounced than in the above species and the exciple is lighter in colour. The margin is often ciliate with free hyaline hyphae. The asci (40–50 × 4.5–6.5 μ) and spores (8–11.5 × 1.5–2.5 μ) are also larger.

Tapesia fusca Fckl. On decayed *Betula* sp., Salmon River, July 3, 1931, leg. A. H. Smith, (364), det. E. K. Cash.

— **melaleucoides** Rehm. On decorticated hardwood, Victoria Park, July 5, 1935, (1725).

Helotiaceae

Chlorosplenium aeruginascens (Nyl.) Karst. On decayed hardwood, New Glasgow Rd., July 25, 1931, (1155); Boularderie Island, Cape Breton Co., Aug. 4, 1931, (1155a). The asci of these collections are 35–48 × 3–5 μ and the spores 6–7 × 1.5–2 μ.

— **aeruginosum** (Oed.) de Not. On rotten wood, Folleigh Lake, Aug. 23, 1927, leg. A. R. Prince, (6039). The asci of this collection are 60 × 7 μ and the spores are 10.5–11.5 × 2–2.5 μ.

— **versiforme** (Pers.) de Not. On rotten wood, Princeport, Colchester Co., Aug. 26, 1927, leg. A. R. Prince, (6158); Earltown Rd., Aug. 21, 1931, leg. A. H. Smith, (1385). Rehm (10, p. 493) gives the spores of his *Coryne versiforme* as 9–14 × 3–4 μ, and the apothecia as 1–3 cm. in diameter. Phillips (8, p. 14) gives the spores of the same species as 8 × 4 μ and the apothecia as "5 lines broad." Collections made by the writer near Chelsea, Mich., agree well with Rehm, being 1–3 cm. in diameter and with spores 10–16 × 2–2.5 μ. Both of the Nova Scotia collections have smaller apothecia (2–8 mm.). No 1385 has spores 10–11.5 × 2–2.5(3) μ and agrees fairly well with Rehm's description. No. 6158 has spores 7–9 × 2.5–3(3.5) μ and fits Phillips' description. Either this species is widely variable, or there are two varieties with different spore sizes.

Dasyscypha Agassizii Berk. & Curt. On *Abies balsamea*, Moore's Lake, Halifax Co., July 6, 1929, (22); Upper Brookside, June 30, 1931, (330); July 13, 1931, (1662). Common on dead bark of fir.

— **occidentalis** Hahn & Ayers. On *Larix laricina*, Upper Brookside, July 8, 1931, (436). This is also close to *D. oblongispora* Hahn & Ayers, but seems to fit better the above species. The apothecia are deep orange, 0.5–1.5 mm. in diameter and thickly scattered. The asci are 96–106 × 8–9 μ and the spores 13–17.5 × 5–7 μ. The paraphyses are very slightly enlarged at the tip, if at all, and are 1.5–2.5 μ in diameter at the apex.

Helotium citrinum (Hedw.) Fr. On dead stem of *Alnus*, Upper Brookside, June 27, 1931, (308).

— **epiphyllum** (Pers.) Fr. On leaves of *Fagus*, Victoria Park, Aug. 17, 1931, leg. A. H. Smith, (1330). On leaves of *Fagus* and *Acer*, Earltown Rd., Aug. 22, 1931, (1330a).

Lachnella corticalis (Pers.) Fr. On bark of *Populus* sp., Salmon River, Aug. 13, 1931, (1299).

— **tricolor** (Sow.) Phill. var. **microspora** Kanouse. On decorticated scar areas of living beech, Upper Brookside, June 27, 1933, (1562), det. B. B. Kanouse.

Lachnum virgineum (Batsch) Karst. On decayed hardwood, Upper Brookside, July 4, 1931, (382). On *Acer spicatum*, Victoria Park, July 5, 1933, (1573). On beech bark, Upper Brookside, July 16, 1929, (41). On *Spiraea* stems, Upper Brookside, July 13, 1931, (497). Common.

Rutstroemia macrosporus (Pk.) Kanouse comb. nov. *Helotium macrosporum* Pk., Ann. Rept. N.Y. State Museum 26: 28. 1874. On decorticated wood, Upper Brookside, July 26, 1929, (96); Folleigh Lake, July 20, 1931, (1090); Mt. Thom, Aug. 15, 1931, leg. A. H. Smith, (1329), det. B. B. Kanouse.

This fungus is common on old decorticated stumps and exposed roots, blackening the substratum. Dr. Kanouse, who kindly examined this material, contributes the

following nomenclatorial revision of the species: "This species was published as *Rutstroemia nebulosa* (Cke.) Kauff. & Kanouse, (Pap. Mich. Acad. 9: 178. 1929). Since then the type of *Helotium macrosporum* Pk. has been examined and found to be identical. Seaver (Mycologia 26: 344. 1934), reinstated the pre-Friesian genus Calycina, and Peck's species, which he placed therein, became *Calycina macrospora* (Pk.) Seaver. Karsten (Myc. Fenn. 1: 12) published the genus Rutstroemia in 1871. According to the rules of nomenclature, this is a valid genus. In it belongs the fungus which has been reported under various names, some of which have been mentioned here. It becomes necessary to make the following new combination, *Rutstroemia macrospora* (Pk.) Kanouse."

Hysteriaceae

Graphis scripta (L.) Ach. On bark of *Abies*, Middle River, Victoria Co., Aug. 5, 1931, (1245).

— **scripta** var. **topographica** (Willd.) Zahlbr. On bark of *Betula*, Upper Brookside, July 16, 1929, (42); July 6, 1931, (405); Folleigh Lake, July 20, 1931, (1107), det. M. L. Lohman. Common on bark of various trees.

Hysterium pulicare (Pers.) Fr. On bark of *Betula*, Folleigh Lake, July 20, 1931, (1107); Salmon River, Sept. 2, 1931, (1459). On *Populus nigra* var. *italica*, Rosebank House, Baddeck, Victoria Co., Aug. 5, 1931, (1225), det. M. L. Lohman.

Hysterographium Mori (Schw.) Rehm. On *Fagus*, Upper Brookside, Aug. 11, 1931, (1276). On *Populus*, Salmon River, Aug. 1, 1931, (1197), det. M. L. Lohman.

Hypodermataceae

Hypoderma rufilabrum (Berk. & Curt.) Sacc. On twigs of *Acer spicatum*, Upper Brookside, July 16, 1931, (1061).

The copy of Rel. Farl. No. 127, in the University of Michigan Herbarium, shows spores (15.5–18 × 2.7 μ) somewhat shorter and more ellipsoid than those in this collection, which has elongate spores 21.5–30 × 3–4 μ, rounded at one end and tapered toward the other. A break in the protoplast, or a septum (?) appears at maturity. Tehon (in litt.) states that this collection is typical of *H. rufilabrum*, however.

Hypodermella nervata Darker. On leaves of *Abies balsamea*, Cheticamp River, Inverness Co., Aug. 6, 1931, (1255) det. M. L. Lohman. Parasitic, forming elongate apothecia along the midrib of dead leaves on living branches.

Lophodermium exaridum (Cke. & Pk.) Sacc. On leaves of *Kalmia angustifolia*, Portapique Beach, Colchester Co., July 22, 1935, (1667).

— **juniperinum** (Fr.) de Not. On *Juniperus communis*, North River Rd., Colchester Co., July 7, 1931, (415), det. M. L. Lohman.

— **melaleucum** (Fr.) de Not. On leaves of *Rhododendron canadense*, New Glasgow Rd., June 30, 1931, (333), det. M. L. Lohman.

— **nitens** Darker. On needles of *Pinus Strobus*, Victoria Park, June 21, 1933, (1663).

— **Piceae** (Fckl.) v. Höhn. On needles of *Picea canadensis*, Rifle Range, Truro, June 19, 1926, (P377), det. M. L. Lohman.

— **Pinastri** (Fr.) Chev. On needles of *Pinus Strobus*, Victoria Park, June 23, 1926, (P370), det. M. L. Lohman.

— **Tumidum** (Fr.) Rehm. On petioles of *Sorbus americana*, Victoria Park, June 23, 1933, (1537).

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THE RELATION BETWEEN FROST RESISTANCE AND THE PHYSICAL STATE OF PROTOPLASM

I. THE PROTOPLASM AS A WHOLE[1]

BY J. LEVITT[2] AND D. SIMINOVITCH[3]

Abstract

The protoplasm of hardy and non-hardy cells was compared with regard to displacement by centrifuging, rounding-up time on plasmolysis, deplasmolysis injury, rate of Brownian movement, resistance to and recovery from distortion by micromanipulation. The conclusions are: (i) When the cells are in the normally hydrated (i.e., turgid) condition there is either no difference in the consistency of hardy and non-hardy protoplasm, or else it is slightly less in the latter; (ii) when the cells are plasmolysed to the same degree (i.e., with a weaker plasmolyte in the case of the non-hardy) there is no difference in consistency; (iii) when the cells are in equilibrium with one and the same concentration of plasmolyte, the protoplasmal consistency of the non-hardy is greater than that of the hardy; (iv) the non-hardy are more susceptible to mechanical injury.

Evidence has recently been produced, on the one hand of an increased viscosity (2, 3) and on the other hand of a decreased viscosity (6) as a result of frost hardening. In the present paper an attempt will be made to clear up the discrepancies, both by a re-examination of the above results and by the addition of new evidence.

It should be recognized at the outset that a discussion of the physical state of protoplasm involves the use of somewhat controversial terminology. Since protoplasm is a non-Newtonian liquid the term viscosity cannot correctly be used. For this reason Seifriz (7) has suggested the term consistency. Both are the opposite of fluidity. In proportion, as the elastic properties (which may be present even in fluid protoplasm) increase, resistance to change of shape is more properly described as elasticity or rigidity rather than viscosity or consistency. But another physical property becomes important in "solid" protoplasm, namely, plasticity or ductility—the property of being deformed without fracture. The ability to prevent a break may also be due to elastic extensibility ("elasticity" in common parlance).

Since all gradations between the "fluid" and "solid" state exist, it is not always easy to decide which term should be adopted. This is especially true if the protoplasm is subjected to dehydration in steps, whereby its liquid properties progressively disappear.

In the following work, several methods of investigating the physical state of protoplasm have been used. The centrifuge, plasmolytic, and Brownian movement methods presumably measure consistency, since all depend on the fluid characteristics of protoplasm. Resistance of cytoplasmic strands to

[1] *Manuscript received June 17, 1940.*
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tear, and of the protoplast to rupture on deplasmolysis, must be more in the nature of ductility or plasticity. Evidence of this is found in the fact that broken strands remain flagella-like, instead of rounding up as would occur in the presence of surface tension forces. Micromanipulation is capable of yielding information about both the fluid and solid characteristics of protoplasm.

Though sound terminology is essential for clarity, from the point of view of frost resistance, it is relatively unimportant whether, for instance, deplasmolysis injury is due to high consistency or low plasticity (or ductility). The important facts are that the condition is physical and that it varies in a definite way with change in temperature and hardiness.

The Centrifuge Method

Kessler (2) demonstrated that the plastids of hardy plants are less readily displaced than those of non-hardy plants. Recognizing the possibility of an altered specific gravity due to the presence or absence of starch in the plastids, he performed the following control experiment. Non-resistant plants were placed in the dark until all the starch had disappeared (six days). The plastids in these starch-free plants proved more easily displaced than those in the untreated. Obviously, this could not be due to an increased specific gravity of the plastids, and Kessler did not attempt to interpret it so (as stated by Scarth and Levitt (6)). In the later paper, Kessler and Ruhland (3) ascribe the result to a change in the protoplasm. In any case, it was impossible to decide from these results whether the difference in starch content was responsible for the more ready plastid displacement in non-resistant than in frost-resistant plants.

In the case of *Hedera*, on the other hand, since both resistant and non-resistant forms contained starch, Kessler and Ruhland assert that this did not influence the displacement. Yet even in this case, unless *Hedera* is an exception, the quantity of starch in the plastids must have been greater in the unhardened plants. Geffken (1) has, in fact, found that *Hedera* shows the usual carbohydrate changes associated with hardiness.

In the case of *Catalpa*, plastid displacement was too rapid to allow the recognition of a difference in rate. On the other hand, when the starch was allowed to disappear (in the dark), a very high speed was necessary to obtain any displacement at all. Even then, the movements proved to be centrifugal in the resistant, and centripetal in the non-resistant. Thus, even in the absence of starch, hardy and non-hardy cells apparently showed differences in the relative specific gravities of plastids and protoplasm.

Kessler and Ruhland produced further evidence in an attempt to prove the validity of the centrifuge results. Narcosis rapidly reduced frost resistance and allowed a more ready displacement of the plastids. In this case, they state, it must have been the protoplasm that was altered, since both sets of plants were without starch and it is therefore due to a difference in viscosity.

Yet even if narcotized plants are less resistant, and at the same time less viscous than the unnarcotized, this fails to prove that the same holds true for plants differing naturally in frost resistance—especially since the lower "frost-resistance" of narcotized plants may be only apparent. On ice-formation, the concentration of narcotic in the protoplasm increases, and death may be due to this rather than to a reduction in true frost resistance.

Since really hardy cells contain starchless chloroplasts, the centrifuge method is applicable only to those that are starchless in the non-hardy condition as well. The cortical cells of *Catalpa* and *Cornus* when dehardened in the laboratory during winter fulfil this condition. Yet the difference in frost resistance in the two states is enormous. Cortical sections from the dehardened twigs were completely killed by freezing at about $-7°$ C.; those from hardy twigs were uninjured by $-30°$ C. (the lowest temperature tried). In both cases the sections were inoculated with ice to insure freezing.

There is one important cellular difference between *Catalpa* and *Cornus*. In the former, the cortical protoplasts all consist of a layer of cytoplasm surrounding a single large vacuole which stains intensely with neutral red. In the latter plant, only half the cortical cells are of this type. The others have *no single large vacuole—neutral red either fails to stain them or at most causes* a faint brownish red coloration. These will be called "avacuolate" as opposed to the other "vacuolate" cells. Both types are shown in Plate I, A.

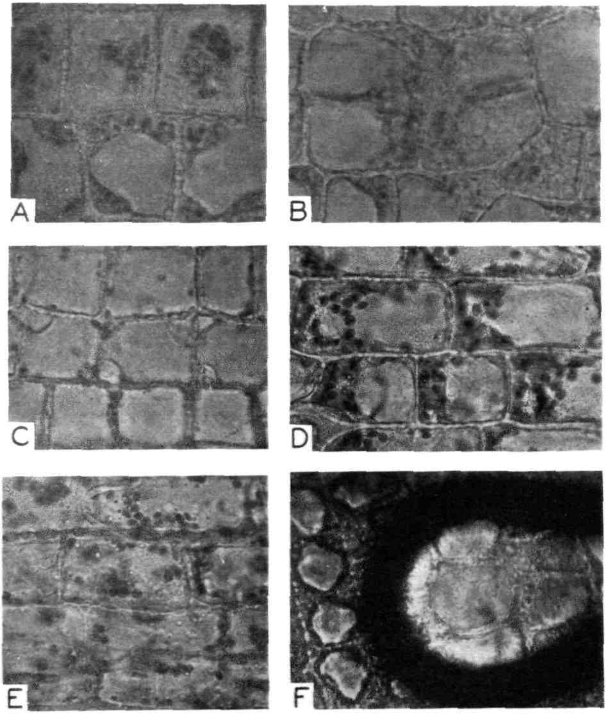
Tangential sections were placed lengthwise in small tubes of water which were inserted in the containers of a hand centrifuge with a radius of either $2\frac{1}{2}$ or 7 in. They were then centrifuged for $\frac{1}{2}$ to 10 min. at 1800 to 4200 r.p.m. and immediately examined. From the known radius, r, and r.p.m., the relative centrifugal force was calculated, using the formula:

$$F = \left(\frac{\text{r.p.m.}}{300}\right)^2 \times r$$

The number 300 was chosen arbitrarily to reduce the otherwise unwieldy figure.

Table I shows that the hardy "avacuolate" cells always permitted readier displacement of their chloroplasts than did the dehardened cells. The difference is clearly revealed in Plate I, B – E. This can be taken as evidence of lower protoplasmal consistency in the hardy than in the dehardened cells only if in both cases (i) the relative specific gravities of the chloroplasts and cytoplasm are the same; (ii) the chloroplast size is the same; (iii) the thickness of the cytoplasm layer is sufficient to permit passage of the chloroplasts. The absence of starch in both cases favours but does not guarantee the fulfilment of all three of these conditions.

In the "vacuolate" cells, however, it is not merely a matter of chloroplast movement. The protoplasm and the vacuole are displaced relative to each other (Plate I, B and D). (It may be noted that hardy *Catalpa* cells showed centripetal as opposed to the centrifugal displacement recorded by Kessler and Ruhland.) Here again relative specific gravities may conceivably be



A. *Cortical cells of hardy Cornus. Upper row "avacuolate", lower row "vacuolate."* *B*. *Cortical cells of hardy Cornus after centrifuging for 2 mins. at 1800 r.p.m. Right centrifugal.* *C*. *Cortical cells of dehardened Cornus after centrifuging for 2 mins. at 1800 r.p.m. Right centrifugal.* *D*. *Cortical cells of hardy Catalpa after centrifuging for 2 mins. at 1800 r.p.m. Right centrifugal.* *E*. *Cortical cells of dehardened Catalpa after centrifuging for 2 mins. at 1800 r.p.m. Right centrifugal.* *F*. *The application of pressure on cortical cells of hardy Cornus by means of a blunt microneedle.*

TABLE I

THE EFFECT OF CENTRIFUGING ON CORTICAL CELLS OF HARDY AND DEHARDENED *Cornus* AND *Catalpa* TWIGS

F = *centrifugal* displacement of *chloroplasts*. *P* = *centripetal* displacement of *protoplasm* as a whole

F (relative centrifugal force)	Time centrifuged, min.	Hardy			Dehardened		
		Cornus			*Cornus*		
		"Avacuolate" cells	"Vacuolate" cells	*Catalpa*	"Avacuolate" cells	"Vacuolate" cells	*Catalpa*
252	2	*F*	*P*	*P*	*O*	*O*	*O*
252	5	*F*	*P*	*P*	*O*	*O*	*O*
252	10	*F*	*P*	*P*	*F*	*P*	*P* (Slight)
360	1	*F*	*P*	*P*	*O*	*O*	*O*
360	4	*F*	*P*	*P*	*O*	*O*	*O*
360	7	*F*	*P*	*P*	*F* (Partial)	*P* (Partial)	*P* (Partial)
490	½	*F*	*P*	*P*	*O*	*O*	*O*
490	10	*F*	*P*	*P*	*F*	*P*	*P*

responsible for the difference between hardy and dehardened cells. In any case, the protoplasm of the hardy cells (and they are *very* hardy) is evidently quite fluid or it would not be so readily displaced in all cases. Consequently these results at least oppose the contention that frost resistance is correlated with high protoplasmal consistency. They may even be interpreted as indicating a greater consistency in dehardened than in hardy cells.

But there are reasons for expecting a difference in the relative specific gravities of plastids, protoplasm, and cell sap in frost-resistant and non-resistant cells. The specific gravities of both protoplasm and cell sap are bound to change with increased frost resistance, owing to the increased hydration of the former (3, 6) and the increased osmotic concentration of the latter. The plastids themselves are modified during winter, apparently independently of the carbohydrate change, for Kraus (4) was able to reverse this change by a one- to two-day exposure to warm temperatures.

Owing to these differences, the centrifuge method is not a reliable test of relative protoplasmal consistency in frost-resistant and non-resistant plants.

The Plasmolytic Method

In the absence of complicating factors, the centrifuge method would reveal the relative consistency of protoplasm in a fully turgid cell. The plasmolytic method, on the other hand, involves a partial dehydration of the protoplasm. It should, therefore, be recognized that even under ideal conditions the two may not give identical results (unless plasmolysed cells are centrifuged), for it is quite conceivable that the dehydration may alter the protoplasmic consistency to a greater degree in one case than in another.

In contrast to the results of Scarth and Levitt (6), Kessler (2) and Kessler and Ruhland (3) recorded a more rapid rounding up of non-resistant than of resistant cells. The latter explain the results of Scarth and Levitt as due to (i) the more rapid evaporation from the less concentrated solution (containing the non-resistant cells) when the evaporation method was used, and (ii) to the Ca^{++} effect. A theoretical consideration of these suggestions is unnecessary, since this was not the only method that yielded these results. At the time when protoplasmic measurements were being made (Scarth and Levitt) it was always more difficult and time-consuming to obtain dehardened cells in the rounded condition, at least in the higher concentrations. Dextrose was the plasmolyte, and no evaporation was involved. However, the sections had previously been vitally stained for half an hour in a weak calcium chloride solution containing neutral red, and for this reason the experiments required repetition. Results with hardened and unhardened cabbage also agree with these, though the differences were relatively small.

The complete absence of electrolytes should eliminate the complication of adhesion (5), provided that there are no naturally occurring polyvalent cations in the cell wall. But plasmolysis shape alone is independable as an indication of relative consistency. Thus both hardy and dehardened *Catalpa* cells, when plasmolysed moderately in dextrose solutions are concave, both hardy and dehardened *Cornus* convex. On the other hand, *Catalpa* cells that have been dehardened for over a month have a very low osmotic concentration and show perfect convex plasmolysis. Hardy *Hydrangea* cells all tend to plasmolyse convexly, but the dehardened cells mostly plasmolyse concavely.

Determinations of rounding-up time, however, should yield some information as to the relative consistencies, provided that in both cases the protoplasts are deformed to the same degree. Since it is not possible to exert much control on the protoplast shape without affecting its consistency, the usual procedure is merely to plasmolyze to the same degree.

Preliminary experiments were performed to test Kessler and Ruhland's criticism. Sections of both hardy and dehardened *Catalpa* were put in hypotonic calcium chloride for some time (one or more hours), after which they were transferred to hypertonic dextrose. Control sections were cut directly into another vial containing the same concentration of dextrose. There was no appreciable difference in the times required for rounding-up, whether or not the sections had been pretreated with the calcium chloride. And this held true both for the hardy and dehardened *Catalpa*. This confirms the value of the results mentioned above.

Comparison of hardy and dehardened cells was made by sectioning the twigs and immediately placing the sections into the plasmolyte (dextrose). *Catalpa* cells are good for this test since both hardy and dehardened plasmolyse with about the same degree of irregularity.

It is seen from Table II that the dehardened were able to round up in two-thirds of the time required for the hardy when both were plasmolysed to about

TABLE II

ROUNDING-UP TIME OF HARDY AND DEHARDENED CORTICAL CELLS OF *Catalpa*

Dextrose conc., *M*	Rounding-up time, hr.	Degree of plasmolysis. (Average of 10 cells)
(a) Dehardened (controls killed by −5° C.)		
0.4	1¾	
.5	2	0.77
.6	2¼	
.7	3	
.8	5	.63
.9	6	.53
1.0	10	.48
(b) Hardy (uninjured by −15 to −20° C.)		
0.9	3	0.72
1.1	4½	.69
1.3	7	.67

three-quarters of their normal value. But the figures reveal that slightly greater volume changes cause disproportionately great increases in time. This is undoubtedly due to the effect of the increased plasmolyte concentration on protoplasmic consistency. If, then, we compare the rounding-up time in the same concentrations of plasmolyte, (i.e., under the same dehydrating conditions) the hardy are found to round up two or more times as fast.

This is in agreement with the results of Scarth and Levitt. When the evaporation method was used, the plasmolyte became highly concentrated and to about the same degree in both cases. (In spite of Kessler and Ruhland's objection, the osmotic pressure of a solution has relatively little effect on its rate of evaporation.) It also confirms the results of Kessler and Ruhland who observed a more rapid rounding up in the case of dehardened cells plasmolysed to two-thirds of their normal volume than in the case of hardy cells plasmolysed to the same degree.

The results do indicate a lower consistency in dehardened than in hardened cells when compared at their isotonic points—as is done when the centrifuge method is used. But this seems only true when the dehardened cells possess a strikingly lower osmotic pressure than the hardened cells. Thus it was shown by Scarth and Levitt that hardy cabbage cells round up more rapidly than do dehardened. These experiments have been repeated and confirmed.

Further evidence of this was obtained with *Catalpa*. A twig, dehardened in the laboratory for a month, had grown two shoots more than an inch long. The osmotic pressure of the cortical cells had dropped to the very low value of 5 to 6 atm. These cells rounded up almost immediately in hypertonic

dextrose solutions. Obviously the protoplasm in equilibrium with this low cell-sap concentration had so high a content of "free" water that its consistency was practically that of water itself. Rounding-up occurred immediately, before the protoplasmic dehydration was sufficient to increase its consistency markedly.

It is apparent from this that the method of rounding-up time is inapplicable when the cells being considered differ greatly in osmotic concentration and non-solvent space. A further complication is in this case introduced. The method assumes that the consistency of the cell sap is negligible in comparison with that of the protoplasm. But in the case of hardy cells it seems certain that the sap is sufficiently concentrated and contains sufficient colloidal matter to endow it with a considerable degree of viscosity. Furthermore, if two cells possess the same protoplasmic consistency, the one with the thinner layer will round up the more rapidly. Hardy cells have thicker protoplasm layers than non-hardy ones, at least in some cases. Finally, it should be remembered that plasmolysis tests involving periods of several hours may not reveal true consistency, owing to changes that may occur during this time. (That a change does occur will be shown in a later section of this paper.)

For these reasons, it is desirable to employ other methods as a check on the results already described.

Deplasmolysis Injury

Scarth and Levitt (6) showed that hardy cells survive deplasmolysis after plasmolysis in strong solutions, while unhardened cells are uninjured only if the plasmolyte is much weaker. They attributed this "deplasmolysis resistance" of hardy cells to a lower protoplasmic viscosity, assuming that the bursting of the cell during deplasmolysis is due to the high consistency either of the protoplasm as a whole or of its membrane. Evidence of this was the fact that the injury increased when the temperature was lowered during the deplasmolysis. Kessler and Ruhland (3) suggest that plasticity rather than viscosity is the true explanation. Since all the above experiments employed a balanced solution of sodium chloride and calcium chloride (9 : 1), it would seem advisable to test the effect of sugars, in order to eliminate any specific ion effect that may differ in the case of hardy and non-hardy cells.

Using dextrose, it was found that unhardened cabbage cells were completely killed on deplasmolysis from a 2.0 *M* solution, and 50% killed from 1.5 *M*. Hardened cells showed 50% killing only when the 2.0 *M* concentration was used. *Catalpa* gave similar results. Naturally hardened cortical cells were killed by deplasmolysis when 4 *M* sucrose was the plasmolyte; dehardened cells suffered the same injury after plasmolysis in 2 *M* sucrose. But the injury in the 4 *M* sucrose was probably not deplasmolysis injury. The high concentration of sucrose tends to cause vacuole contraction rather than plasmolysis, and this abnormal reaction is in itself injurious. And since the tests were made in December, the cells had not yet reached their maximum degree of hardiness. When tested later in the winter, using solutes that do

not cause vacuole contraction (dextrose or a balanced solution), no deplasmolysis injury was obtainable with the highest concentrations (e.g., 6 *M* dextrose), even though these possessed greater osmotic values than the 4 *M* sucrose.

In the case of cabbage, the increase in concentration required to produce the same injury in hardy as in non-hardy cells was probably just sufficient to counterbalance the cell sap differences. In other words, the cell contraction in the plasmolytes was about the same in the two cases. Deplasmolysis injury, however, must depend not only on the degree of expansion the cell is exposed to, but also on the physical state of the protoplasm. It would, therefore, seem that the hardy cell had about the same protoplasmic consistency in the more strongly dehydrating solution as the non-hardy one in the less strongly dehydrating solution. This difference between hardy and non-hardy cells is obviously less than that previously obtained. The explanation of the apparent discrepancy will appear in Part II of this series.

Brownian Movement

Scarth and Levitt were unable to detect any difference in the rate of movement of particles in hardened and unhardened cabbage. In hardy *Catalpa* no movement was seen. A re-examination of the latter has revealed the reason for this. The hardy *Catalpa* cell (as seen from above) is surrounded by a broad ring of protoplasm. Observation of this peripheral zone involves a view of a thick layer filled with plastids and various particles. It would be very difficult to see any movement here. If, however, the microscope is focused on the upper surface of the protoplast, a relatively thin and transparent layer is viewed and the number of particles seen is much less. These are clearly in Brownian movement, and even streaming.

Cortical cells of both hardy and dehardened *Catalpa* and *Hydrangea* were observed. The hardy twigs were taken indoors in April and placed in a cold chamber at about 0° C. At about the time that observations were made, some twigs were frozen at temperatures down to −17° C. without suffering the slightest injury. They all sprouted within about a week of this. (To insure freezing, the cut ends of the twigs were standing in a layer of distilled water.)

The dehardened twigs were collected in April, and kept in water in the laboratory for two or more weeks. When tested, they all had green young sprouts. Twigs frozen in the cold chamber at −5° C. proved to be dead several days later.

Instead of attempting to distinguish a difference in rate of movement, which is very difficult and highly subjective, the sections were placed in solutions of different concentrations, and the concentration capable of stopping the movement was determined. A concentration of 0.50 to 0.75 *M* dextrose was sufficient in the case of dehardened *Catalpa* and *Hydrangea*. Hardy *Hydrangea* required 1.0 to 1.5 *M*, hardy *Catalpa* 1.5 to 2.0 *M*.

Here again, the hardy cells proved to have a lower consistency than the dehardened when in equilibrium with the same dehydrating forces.

Micromanipulation

This method investigates the relative resistances to distortion offered by protoplasm in hardy and dehardened cells. Tangential cortical sections of *Catalpa* or *Cornus* twigs were mounted in a hanging drop of a balanced solution (sodium chloride and calcium chloride in the molar ratio of 9 : 1). One micro-needle held the section rigidly in place, while the other was left free for manipulation.

The thin pits in the otherwise thick walls offered an easy locus for the penetration of the needle, but only when the cells were plasmolysed. Plasmolysis was also necessary for observation of the effects of manipulation. Frequent renewal of the solution compensated for evaporation during manipulation.

Three main methods were used for investigating the relative consistencies of hardy and dehardened cortical cells of *Catalpa* (Methods 1 and 2) and *Cornus* (Method 3).

(1) The protoplasts were manipulated in two ways: a furrow was made in the protoplasm and recovery after removal of the needle was noted; or pressure was exerted on the wall, causing distortion of the protoplast, the release of pressure permitting reversion to its original shape. In both cases relative fluidity could be estimated by the ease (i.e., the speed) with which the protoplasm was able to change its shape.

In all cases an increase in consistency occurred with increased dehydration. In a balanced solution with an osmotic concentration of about 50 atm., the protoplasm of hardy cells flowed easily and responded quickly to pressure, resembling a thin, sticky glue. The protoplasm of dehardened cells behaved like a viscous, flowing plastic. But the osmotic concentration of hardy cells (20 atm.) is about double that of dehardened ones. A comparison was therefore made using $2\frac{1}{2} \times$ isotonic solutions for each (i.e., with osmotic concentrations of 50 and 25 atm. respectively). In this case there was no apparent difference in consistency between the protoplasm of hardy and dehardened cells. Similarly, a solution with an osmotic concentration of 100 atm. for hardy cells and one with an osmotic concentration of 50 atm. for dehardened cells, failed to reveal a difference. When the concentrations were again doubled (200 and 100 atm. respectively), the consistency in both cases was such as to allow almost permanent molding into any shape.

Manipulation caused coagulation of the protoplasm and puncturing of the vacuole much more frequently in dehardened cells than in hardy ones.

(2) In the second method, a pit in the wall was punctured and the protoplast squeezed out of the hole by pressure on another part of the wall. The shape of the extruded mass was taken as an indication of relative consistency. By first staining with neutral red, it was possible to observe that the protoplasm and not the vacuole was extruded in all the cases discussed. The results are shown in Table III and Fig. 1.

TABLE III

SHAPE OF PROTOPLASMIC EXTRUSIONS FROM HARDY AND DEHARDENED CORTICAL CELLS OF *Catalpa*

	Osmotic concentration (atm.) of balanced solutions		
	25	50	100
Hardy	Spherical	Pear-shaped	Narrow tongue with irregular thickenings
Dehardened	Spherical→pear-shaped	Narrow tongue with irregular thickenings	Could not be squeezed out

If deplasmolysis was permitted, a spherical extrusion occurred from both hardy and dehardened cells. In the dehardened, however, only a slight expansion occurred before the extruded mass burst. In the hardy, the extruded mass burst only after expanding to fully the size of the cell itself.

As in the first method, the hardy protoplasm showed a much greater tolerance toward micromanipulation than did the dehardened. It could be extruded and retracted again and again, and even torn away and another mass of protoplasm extruded without any coagulation. The dehardened protoplasm on the other hand, usually coagulated at the first extrusion. Consequently only a minority of the cells could be used—cells with protoplasm having an apparently reduced viscosity as judged by the increased vacuolation.

(3) In the third method, a more or less uniform pressure was applied to the cells by means of the well-rounded end of a blunt micro-needle (Plate I, F). Both the ease of deformation and the speed of readjustment were noted.

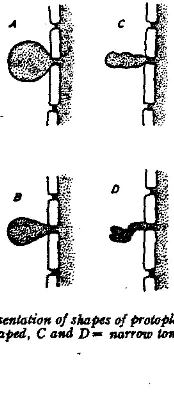


FIG. 1. *Diagrammatic representation of shapes of protoplasm extrusions from a punctured pit. A = spherical, B = pear-shaped, C and D = narrow tongue with irregular thickening.*

In solutions of similar relative osmotic concentrations (25 atm. for the dehardened, 50 atm. for the hardy) consistency was about the same in the dehardened and hardy cells. In solutions with 4 × these concentrations (100 atm. and 200 atm. respectively), the protoplasts retained their flattened configuration after the pressure was released. In solutions with the intermediate concentration (100 atm.) the hardy protoplasts were still able to resume their original shape after release of pressure, though not as quickly as in a solution of 50 atm. concentration. In solutions with osmotic concentrations of 400 atm. and greater, the protoplasm was apparently a solid, for it fractured as a result of pressure, allowing escape of the vacuole contents.

Hardy protoplasts expanded readily under such pressures, sprang back to their original shape on release of the pressure, and in most cases were even capable of deplasmolysis. They were injured only if the pressure was great enough to cause a sudden extrusion through a burst pit. Dehardened cells, however, were killed long before expansion to the degree permitted by hardy cells. Sometimes the dehardened protoplast "exploded", as though it had reached the limit of its extensibility.

All these micromanipulation results indicate that:

(1) When the cells are in the unplasmolysed condition there is no appreciable difference between the protoplasmal consistency of hardy and dehardened cells.

(2) When the cells are in equilibrium with hypertonic solutions bearing the same relation to the isotonic value of the respective cells (about twice as great in hardy as in dehardened *Catalpa*), there is also little difference between hardy and dehardened.

(3) When the cells are in equilibrium with one and the same concentration of plasmolyte, the protoplasmal consistency of the dehardened is greater than that of the hardy. The difference increases with the concentration of the solution.

(4) When the hardy and dehardened cells are compared under any one of the above three sets of conditions, the dehardened are more susceptible to mechanical injury.

But how do these conclusions agree with those arrived at by means of the other three methods?

(1) Results with the centrifuge method prove that hardy protoplasm is fluid in turgid cells. They might also be interpreted as indicating a higher consistency in dehardened than in hardy cells, were it not for the complication due to differences in relative specific gravities of protoplasm, plastids, and cell sap. Results with the rounding-up time technique indicate the opposite—a *lower* consistency in dehardened than in hardy cells, but only when the cell sap concentration of the dehardened is much lower than that of the hardy, and the protoplasm is therefore in equilibrium with a much lower dehydrating force. It is quite possible that the micromanipulation method may be incapable of detecting differences in consistency between two systems in this

highly fluid state. The results obtained with the Brownian movement method throw no light on this point.

(2) Results obtained with the plasmolysis-time method indicate a higher consistency in the hardy cells. But this evidence cannot be considered as conclusive since the rounding-up time (five to seven hours) was long enough for the occurrence of protoplasmic changes (see later section). Results with the deplasmolysis method, however, agree with those obtained by micromanipulation.

(3) The centrifuge method does not deal with this point. *All four remaining methods agree.*

(4) This point does not throw any light on the consistency of the protoplasm as a whole, but as will be shown in Part II of this series, depends on the rigidity of the ectoplasmic layer.

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VARIABILITY IN ASSOCIATION EFFECTS OF OTHER SOIL FUNGI ON THE VIRULENCE OF *HELMINTHOSPORIUM SATIVUM* ON WHEAT SEEDLINGS[1]

By G. B. Sanford and M. W. Cormack[2]

Abstract

Random isolates of *Penicillium*, *Actinomyces*, and certain miscellaneous soil-inhabiting fungi were tested in steam sterilized soil, under pure culture conditions, for their association effects on the virulence of *Helminthosporium sativum* P. K. and B. on wheat seedlings. Certain isolates of the first two genera mentioned exerted a marked degree of suppression, some had no effect, while others increased the virulence. Similarly, these effects varied widely within certain species of *Penicillium*. This preliminary study indicates that the random isolates of many genera and species of fungi may differ widely in ability to affect the virulence of certain plant pathogens.

The ability of various bacteria and fungi to reduce the virulence of certain plant pathogens in soil culture has been established. However, no one seems to have determined the diversity in this connection that may exist within a given genus or species.

Following is a preliminary report of experiments, carried out under pure culture conditions, to demonstrate the range of antagonism exhibited by 24 isolates of *Penicillium* spp., eight isolates of *Actinomyces* spp., and several miscellaneous fungi, to the virulence of *Helminthosporium sativum* P. K. and B. on wheat seedlings.

Methods

In 1933, dilutions from composite samples of soil from four wheat fields near Edmonton, Alberta, were plated out in suitable media. The numerous isolates of fungi secured were differentiated by means of cultural and microscopic studies, and types that occurred most frequently in each soil were selected for the present study. Single spore cultures were used in all experiments. Dr. G. R. Bisby, Imperial Mycological Institute, Kew, England, and Dr. J. E. Machacek, Dominion Laboratory of Plant Pathology, Winnipeg, kindly identified a number of the isolates listed in Table I.

A technique was developed for growing wheat seedlings aseptically in wide-mouthed, one-litre, Erlenmeyer flasks with only the pathogen and one associate present. All flasks contained 300 gm. of a 3 : 1 soil-sand mixture of uniform moisture content. The flasks, each stoppered with a special cotton plug containing a circular wire frame and a wire stirring rod, were autoclaved twice for a period of four hours on successive days at 15 lb. pressure. As this special stopper was not removed until the end of the experiment, the chances

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for contamination were practically eliminated. All materials were introduced aseptically through a small hole in the centre of the cotton plug. This hole, which was supported by the wire frame mentioned, was plugged with cotton.

Standardized spore suspensions consisting of the pathogen only, of the pathogen with the particular associate to be tested, and of each of the associates only, were prepared and 50 cc. of the given spore suspension was pipetted into each flask as required. Four flasks were used for each treatment.

Marquis wheat seed was soaked for three hours in water and then placed in a 1 : 1,000 solution of mercuric chloride for seven minutes. This method of seed disinfection proved most satisfactory for producing sterile seedlings. Following the application of the various spore suspensions indicated, 20 of these treated seeds were put into each flask, then distributed and slightly buried with the stirring rod. A layer of fairly dry, sterile sand was then spread over the planted surface to a depth of one-half inch. This sand prevented the mycelium of the pathogen from growing on the surface and attacking the seedlings above the soil line.

The water content of the soil, when the seeds were planted, was ideal for good plant growth, and water was not added during the incubation period. Plating tests showed that practically all flasks remained uncontaminated at the end of the experiment. All contaminated ones were discarded. Final notes on germination, height of plants, and infection were taken at the end of three weeks. The three groups of fungi mentioned were tested at different times.

Results

Penicillium spp.

Penicillium spp. were isolated much more frequently than any other fungus. Therefore, a relatively large number of these random isolates were examined for association effects on *H. sativum*. The effect of each isolate on wheat seedlings with the pathogen absent was also determined. When the isolates were associated with *H. sativum* the virulence of this pathogen, as indicated by the infection ratings in Table I, ranged from 16 to 97%, in comparison to 73% for *H. sativum* alone. *P. simplicissimum* (Oud.) Thom, and *P. fuscus* Sopf., depressed this rating by 57 and 42%, respectively. One isolate of *P. chrysogenum* Thom, depressed virulence by 39%, while another increased it by 20%. One isolate of *P. expansum* Thom, depressed virulence 31%, while two others indicated very little, if any, effect. An isolate of *P. glabrum* (Wehmer) Westling depressed the virulence by 24%, and two isolates of *P. terrestre* Jensen by 18 and 11%, respectively. *P. Trzebinskii* Zaleski, had no effect and *P. rugulosum* Thom increased it by 22%. The four isolates of *P. viridicatum* Westling indicated wide diversity in its effect upon virulence of the pathogen; two reduced it by 22 and 17%, respectively, one had no definite effect, while the fourth increased virulence by 11%. One isolate of *P. chloroleucon* Biourge depressed the virulence by 17%, and another had no effect. Two isolates of *P. cyclopium* Westling had no definite effect. Of the

TABLE I

THE EFFECT OF RANDOM ISOLATES OF CERTAIN SOIL FUNGI ON THE VIRULENCE OF *Helminthosporium sativum* ON WHEAT SEEDLINGS

Isolate	Dead plants	Av. ht., cm.	Av. rt.,[1] %	Isolate	Dead plants	Av. ht., cm.	Av. rt.,[1] %
Penicillium				*Miscellaneous fungi*			
P. simplicissimum	0	23.4	16	*Trichoderma viride*	0	23.1	4
P. fuscus	1	20.3	31	*T. lignorum*	0	23.0	6
P. chrysogenum	1	22.8	34	*T. lignorum*	0	23.9	12
P. expansum	3	20.0	42	*Absidia glauca*	2	22.3	23
P. glabrum	7	19.1	49	*Aspergillus nidulans*	10	18.7	32
P. viridicatum	13	18.9	49	*Mucor hiemalis*	19	20.6	57
P. terrestre	5	19.0	55	*Rhizopus* sp.	18	22.2	55
P. chloroleucon	22	21.6	56	*Rhizopus* sp.	18	16.6	62
P. viridicatum	6	21.9	56	*Aspergillus niger*	22	20.1	65
P. terrestre	14	18.0	62				
Penicillium[2]	16	21.0	62	*H. sativum* alone	13	18.4	62
				Wheat alone[3]	0	22.0	0
P. expansum	13	14.0	69	Fungi alone[4]	0	22.7	0
P. viridicatum	24	18.3	70				
Penicillium[2]	23	18.8	71	*Actinomyces*			
P. chloroleucon	21	18.0	72				
P. cyclopium	22	17.9	73	*Actinomyces* 2	0	22.3	33
P. Trzebinskii	23	17.6	73	*Actinomyces* 3	12	24.6	44
P. expansum	23	16.4	75	*Actinomyces* 6	12	24.9	44
Penicillium[2]	23	16.9	78	*Actinomyces* 7	17	25.2	55
P. cyclopium	22	11.9	78				
Penicillium[2]	19	13.7	81	*Actinomyces* 5	23	28.0	65
				Actinomyces 4	25	25.9	70
P. viridicatum	29	12.7	87				
P. rugulosum	37	11.1	95	*Actinomyces* 1	29	24.5	78
P. chrysogenum	38	9.0	97	*Actinomyces* 8	26	22.9	82
H. sativum alone	22	18.1	73	*H. sativum* alone	21	22.3	66
Wheat alone[3]	0	22.2	0	Wheat alone[3]	0	24.2	0
Isolates alone[4]	0	20.3	0	Isolates alone[4]	0	26.4	0

[1] *Disease rating.*
[2] *Species not identified.*
[3] *No micro-organisms present in soil.*
[4] *Average effect of isolates as tested separately, and the pathogens absent.*

four remaining unidentified isolates one depressed virulence by 11%, two had no definite effect, and one increased virulence slightly.

Miscellaneous Fungi

Of the miscellaneous fungi tested, the depressing effect of *Trichoderma* spp. on the virulence of *H. sativum* (Table I) was very great. An isolate of *T. viride* (Pers.) ex Fries, reduced the infection rating by 58%, and two of *T. lignorum* (Tode) Harz. reduced it by 56 and 50%, respectively. A random isolate of *Absidia glauca* Hagem, reduced infection 39%, and one of *Aspergillus nidulans* (Eidam) Wint. by 30%. The effect of *A. niger* v. Tieg., *Rhizopus* sp., and *Mucor hiemalis* Wehm., was not appreciably different from that of *H. sativum* alone.

Actinomyces spp.

In comparison with a disease rating of 66% for *H. sativum*, four of the eight isolates of *Actinomyces* depressed the virulence of the pathogen by 33, 22, 22, and 11%, respectively; two had no marked effect; and the other two appeared to increase it by 12 and 16%, respectively (Table I).

Discussion

The foregoing experiments have demonstrated that a fairly high percentage of random isolates of the genus *Penicillium* are quite antagonistic to the virulence of *H. sativum* on wheat seedlings, and also that certain random isolates within a given species may be very antagonistic, while others are not. Other work has shown that certain isolates of *Penicillium* reduced the virulence of *Pythium debaryanum* (5) and of *Ophiobolus graminis* (3, 4). Asthana (1) found that, of several fungi used, *Penicillium chrysogenum* was among those that exerted the greatest repressive effect on the growth and parasitic vigour of *Botrytis cinerea*. It is obvious from the present studies that within a given species some isolates may exert no noticeable effect on the virulence of the pathogen, others may depress it, and some actually increase its virulence. Possibly this ability to increase the virulence of a pathogen is even more common among micro-organisms than would appear from the data presented. An increase of virulence of *O. graminis* by the action of certain associated fungi was reported in 1931 by Sanford and Broadfoot (4), and Borzini (2) reported a similar case in 1937.

Obviously it is important to know the relative antagonistic effect on a given pathogen of the various genera and species of fungi that inhabit the soil, but it would appear from these studies that it is even more essential to ascertain the range of variation of this kind exhibited by random isolates of a given species. In other words, the behaviour of a single isolate may be quite different from the average for the species. The interesting question now is whether the plant pathologist, in controlled greenhouse or ordinary field experiments, is more concerned with the average association effects of a given species than with those of particular isolates or groups of them. At least it is reasonable to suppose that in rigidly controlled experiments such as are reported here there would be instances where the effects of one isolate counterbalance those of another, and that in natural soil the combined effects of other micro-organisms might produce a different result. It is regretted that more complete data on this general question cannot be supplied at this time.

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VEGETATIVE PROPAGATION OF CONIFERS

VII. OUTDOOR PROPAGATION OF A NOVEMBER COLLECTION OF NORWAY SPRUCE CUTTINGS TREATED WITH PHYTOHORMONES, CANE SUGAR, AND AN ORGANIC MERCURIAL DISINFECTANT[1]

By N. H. Grace[2], J. L. Farrar[3], and J. W. Hopkins[4]

Abstract

Dormant Norway spruce cuttings were collected in mid-November and treated with talc dusts containing two separate phytohormone chemicals, indolylacetic and naphthylacetic acids, in three concentrations, 0, 1000, and 5000 p.p.m., alone, and in combination with cane sugar and an organic mercurial disinfectant. Treated cuttings were planted immediately in outside frames in sand and a mixture of sand and peat in equal proportions. They were removed for examination 10 months later. Phytohormone treatment, except with the 5000 p.p.m. concentration of naphthylacetic acid, which was injurious, increased the number of cuttings rooted, the number and length of roots, the number of surviving cuttings, and the number of rooted cuttings with new growth. There were also marked effects on the initiation and development of new growth. The admixture of peat in the propagation medium improved rooting and development of new growth, particularly in certain of the phytohormone treatments. Only about 50% of the controls rooted in both sand and sand-peat, while the 5000 p.p.m. concentration of indolylacetic acid effected 68% rooting in sand and 82% in sand-peat.

Both cane sugar and organic mercury significantly affected a number of the responses. However, the effects were comparatively small and depended, for the most part, on interactions with phytohormone treatments and media. Organic mercury increased the number of cuttings rooted by about 6%.

Introduction

Although special consideration has been given to the rooting of cuttings of Norway spruce in the course of studies on the vegetative propagation of conifers by several workers (3, 6, 8, 11, 14, 18), much of the experimental work so far reported has dealt with propagation in the greenhouse. While this method of approach may contribute valuable physiological information, it also has to be recognized that methods of propagation in outside frames must be perfected before vegetative propagation can be applied in forestry on any large scale.

This communication accordingly describes an experiment in which dormant Norway spruce cuttings were taken in November and planted in outside frames. The experiment involved the use of a series of dust preparations containing plant hormones and other chemicals previously shown to have some influence on the rooting of cuttings (7, 9). The dust method of applying

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the chemicals was chosen in preference to the solution method, which has failed, in the experience of the authors, to have beneficial effects on the rooting of cuttings of this species of spruce (3, 4, 10, 11). The experiment was made in two media, sand and a mixture of 50% sand and 50% peat humus by volume, and was designed to permit consideration of the effects of the various chemicals individually, in combination, and in relation to the two media employed.

As a previous paper has already dealt with similar Norway spruce cuttings collected at the same time and treated with the same dusts, but propagated during the winter in a greenhouse (9), it is also possible to draw some broad comparisons between outside and greenhouse methods of propagation of cuttings of this species.

Experimental

Branches were collected from the upper half of Norway spruce trees 18 years old in a plantation situated at the Petawawa Forest Experiment Station, Chalk River, Ontario. Cuttings without a heel of old wood, and ranging from 2 to 3.5 in. in length, were rapidly taken with secateurs from twigs less than 8 in. long. Each group of 30 cuttings, the unit in the experiment, was representative of the different lengths. There were approximately four lateral cuttings to one terminal. Preparation, treatment, and planting of the cuttings was completed on November 15, 1938, the day the branches were obtained.

The series of dust preparations (9) contained the two phytohormones indolylacetic and naphthylacetic acids, each separately, at three concentrations, namely, 0, 1000 and 5000 p.p.m. (p.p.m. of hormone in hormone talc mixture), in conjunction with cane sugar at 0 and 10% and ethylmercuric phosphate at 0 and 50 p.p.m., all in talc. There were thus 20 different preparations in this factorial series of dusts. The freshly prepared cuttings were dipped without dampening to a depth of about $\frac{3}{4}$ in. into the appropriate dust in groups of 30 and planted in four replicate randomized blocks. Each of the four replicate blocks also contained four duplicates of the treatments with talc, sugar, and organic mercury, in order to provide averages for cuttings with no phytohormone treatment of the same weight as those for each hormone dosage when averaged over both chemicals. The entire experiment required 2880 cuttings.

Two contiguous 3.5 by 24 ft. propagation frames shaded by screens of lath covered with factory cotton were used. One accommodated two complete replications planted in sand, and the other, two replications in the mixture of sand and peat humus.

The sand employed was fine and quite uniform, 91% passing through a 1 mm., and being held by a 0.05 mm., sieve. Its clay fraction was about 2% and organic matter content less than 1%. A volume of 100 cc. held 27 cc. of water against gravity. The reaction of the sand was slightly acid, with pH ranging from 6.2 to 6.5.

The peat humus was obtained from Alfred, Ontario, through a commercial supply house. The products of this peat bog have been described elsewhere (12, 17). Analysis of the shipment used in this experiment showed the ash content to be 10.5%. About one-quarter of the ash was lime but only half of it was classified as exchangeable. Qualitative tests showed that nitrates were present but not nitrite or ammonia. Nitrifying bacteria were shown to be present, and the pH was found to be 6.8*. Because of its fine texture the peat humus mixed readily with the sand. A volume of 100 cc. of the mixture held 35 cc. of water against gravity.

Cuttings were planted at a depth of about 1.5 in. and were left in the frames continuously until mid-September, 1939, when they were removed for counts and measurements. From the date of planting until early in June they received no attention. After that date the frames were watered daily, the sand being maintained at a usual moisture content of 4 to 7 cc. and the peat-sand at 22 to 30 cc. per 100 cc. of medium. As the pore space per 100 cc. of medium was 40 cc. for sand and 67 cc. for the mixture of sand and peat, this left an air content of about 35 and 41 cc. respectively in the two media.

The air temperature in the frames rarely fell below 50° F. or rose above 100° F. during June, July, and August. The maximum temperature reached in the frame was 14° above that outside. Evaporation ranged from 20 to 60 Livingston units per week (16). This was about 20% of the evaporation from an exposed atmometer in the same locality, and about half of that recorded in a greenhouse at New Haven (3, 4). The composition of the medium had no apparent effect on air temperature or evaporation.

While imported granulated peat moss has been used by various workers in the propagation of cuttings, the domestic sedge peat employed in this experiment has been used by comparatively few (1, 13, 15, 19). The addition of any peat to sand will increase the amount of moisture held, and it is quite possible that this is an important effect. Peat also affects the aeration, acidity, and base exchange capacity. Its effect may also be due to its chemical constituents or to the microflora it contains.

Results

Statistical Treatment of the Data

In Table I are given the results of the analyses of variance of the counts and measurements made on the cuttings. Counts of the number of cuttings with new growth, terminal new growth, and the number of new growth shoots per group of 30 cuttings were made in early August and the data analysed as such. Data for the number of cuttings surviving at that time were subjected to the inverse sine transformation prior to analyses of variance (2), as were all counts made in September, when the cuttings were taken up. Succeeding tables illustrate, not all the statistically significant differences, but a selection of the more important effects and interactions of the chemical treatment and propagation medium.

* *The analyses on the peat humus used in this experiment were carried out by Dr. C. C. Heimburger, Dominion Forest Service, Ottawa.*

TABLE I

ANALYSIS OF VARIANCE OF RESPONSES OF *NORWAY SPRUCE CUTTINGS* TREATED WITH TALC DUSTS CONTAINING PHYTOHORMONES, CANE SUGAR, AND ORGANIC MERCURY, AND PROPAGATED IN SAND AND SAND-PEAT

Source of variance	Degrees of freedom	Mean square										
		Number of cuttings rooted	Number of roots per cutting rooted	Length of root per rooted cutting	Mean root length	Number of surviving cuttings		Number of cuttings with		Number of new growth shoots per group of cuttings	Number of rooted cuttings with new growth	Per cent of rooted cuttings (without new growth—with new growth)
						August	September	New growth	Terminal new growth			
Replicates												
Between media	1	1123.5	0.41	417.9	31.21	92.6	2.5	442.0***	615.1***	1683.4***	4198.9*	12398.8*
Within media [Error (*a*)]	2	79.1	2.76	28.7	3.79	20.6	35.5	2.2	6.5	0.9	82.2	654.0
Sugar	1	191.0	0.03	5.0	0.20	297.2*	516.2*	88.2**	106.3**	216.0**	229.1**	19.3
Mercury	1	238.1*	7.21*	93.4**	0.88	75.1	26.5	3.4	0.3	45.4	132.3*	250.3
Sugar × mercury	1	39.8	2.13	0.8	0.97	77.9	15.7	28.2	14.3	26.0	145.3*	250.3
Phytohormone												
Dosage	2	507.8**	4.12*	12.1	0.35	1091.4***	2450.4**	382.6***	335.7***	503.6***	304.0***	6598.4***
Chemical	1	5672.0**	14.34**	59.1**	0.14	2907.9***	9560.0**	915.1***	708.9***	2743.2***	3284.7***	8649.0***
Chemical × dosage	1	5781.7**	40.6	85.3*	1.27	1260.3***	5073.0**	400.0***	319.5***	791.0***	2741.8***	1936.0
Phytohormone × sugar												
Dosage × sugar	2	6.7	1.18	2.7	0.07	479.1**	600.9**	10.4	6.6	44.0	32.2	659.1
Chemical × sugar	1	209.2	1.79	0.4	0.06	173.6	141.6	52.6*	40.6	107.6*	123.5	3.1
Dosage × chemical × sugar	1	614.4**	0.58	0.1	0.03	208.8	329.4*	0.3	0.1	34.5	147.9*	315.1
Phytohormone × mercury												
Dosage × mercury	2	143.4	0.62	1.7	0.25	317.1**	192.0	2.4	1.5	27.8	14.7	260.0
Chemical × mercury	1	38.9	1.13	26.7	0.74	15.1	355.3*	0.6	5.6	26.3	29.6	529.0
Dosage × chemical × mercury	1	53.5	1.65	0.9	0.06	26.0	0.5	2.3	5.6	43.9	0.9	4.0
Phytohormone × sugar × mercury												
Dosage × sugar × mercury	2	145.9	4.11*	5.8	0.30	26.5	37.9	9.3	13.0	14.5	186.3**	2351.1*
Chemical × sugar × mercury	1	0.4	3.56	0.6	0.46	76.1	141.0	4.0	3.5	23.8	0.4	1388.8
Dosage × chemical × sugar × mercury	1	16.3	2.15	24.9	2.81**	39.7	44.6	15.6	0.8	54.4	12.9	661.8
Sugar × media	1	175.5	1.58	2.2	0.13	19.7	105.8	6.0	11.4	22.0	404.7**	810.8
Mercury × media	1	72.8	0.21	50.6	1.34	37.6	160.7	1.0	10.0	16.7	38.4	86.3
Sugar × mercury × media	1	7.6	1.15	4.3	1.34	152.3	235.2	8.2	11.3	0.7	4.6	162.8

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

TABLE I—*Concluded*

ANALYSIS OF VARIANCE OF RESPONSES OF NORWAY SPRUCE CUTTINGS TREATED WITH TALC DUSTS CONTAINING PHYTOHORMONES, CANE SUGAR, AND ORGANIC MERCURY, AND PROPAGATED IN SAND AND SAND-PEAT—*Concluded*

Source of variance	Degrees of freedom	Mean square										
		Number of cuttings rooted	Number of roots per cutting rooted	Length of root per rooted cutting	Mean root length	Number of surviving cuttings		Number of cuttings with		Number of new growth shoots per group of cuttings	Number of rooted cuttings with new growth	Per cent of rooted cuttings (without new growth—with new growth)
						August	September	New growth	Terminal new growth			
Phytohormone × media												
Dosage × media	2	624.1**	9.21**	67.1**	0.08	242.8*	408.4**	3.6	7.5	2.8	69.8	2312.3*
Chemical × media	1	0.1	11.46**	0.8	1.54*	258.5*	367.7*	100.0**	102.5**	23.8	79.9	4.0
Chemical × dosage × media	1	76.4	10.33**	52.0	0.06	119.9	42.6	3.1	17.0	62.0	13.4	225.0
Phytohormone × sugar × media												
Dosage × sugar × media	2	68.5	4.79*	18.3	0.07	48.6	23.4	4.9	4.0	5.0	24.9	372.8
Chemical × sugar × media	1	4.4	9.66**	61.4*	0.27	8.1	0.8	1.0	1.9	0.8	40.5	1139.1
Dosage × chemical × sugar × media	1	45.4	2.03	1.0	1.19	62.8	0.0	1.6	5.6	15.0	21.5	162.6
Phytohormone × mercury × media												
Dosage × mercury × media	2	78.6	1.96	28.1	0.03	61.3	2.9	1.0	1.1	4.2	78.1*	542.5
Chemical × mercury × media	1	165.4	0.00	1.7	0.12	2.7	359.1*	6.3	1.9	0.1	38.9	100.0
Dosage × chemical × mercury × media	1	28.2	0.40	0.4	0.00	86.0	13.7	10.6	15.0	1.9	9.5	256.0
Phytohormone × sugar × mercury × media												
Dosage × sugar × mercury × media	2	129.7	1.14	18.7	0.16	98.2	214.2	8.5	7.0	45.5	101.9*	345.2
Chemical × sugar × mercury × media	1	147.3	0.24	0.3	0.15	51.5	189.8	2.3	0.4	1.9	116.9*	297.6
Dosage × chemical × sugar × mercury × media	1	62.6	1.13	65.4*	0.87	71.0	72.6	5.1	8.3	19.2	40.5	39.1
Error (b)	54	56.1	1.12	13.0	0.37	61.1	72.7	10.3	10.9	24.0	24.6	545.6

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

Number of Cuttings Rooted

Table II shows the average effects of phytohormone dust treatments and media on the number of cuttings rooted. The results given are averages for treatment with and without cane sugar and organic mercury. In sand, the 5000 p.p.m. concentration of indolylacetic acid increased rooting whereas this concentration of naphthylacetic acid markedly reduced it. The 1000 p.p.m. concentration of both hormones was without effect. In the mixture of sand and peat, rooting of the controls was of the same order as in sand only. However, each treatment resulted in substantially better rooting in the mixture than did the same treatment in sand. Both concentrations of indolylacetic acid and the 1000 p.p.m. concentration of naphthylacetic acid increased the number of cuttings rooted. The 5000 p.p.m. concentration of naphthylacetic acid reduced rooting, but not to the same extent as in sand. The 5000 p.p.m. level of indolylacetic acid—the best treatment—effected 82% rooting in sand-peat, while the controls without hormone rooted about 48%.

TABLE II

AVERAGE EFFECTS OF PHYTOHORMONE DUST TREATMENTS AND MEDIA ON THE NUMBER OF CUTTINGS ROOTED

Medium	Data	Phytohormone concentration in talc, p.p.m.				
		0	Indolylacetic acid		Naphthylacetic acid	
			1000	5000	1000	5000
Sand	Transformed	46.8	42.8	56.1	44.8	15.6
	Per cent	52.9	46.3	68.3	49.6	11.7
Sand-peat	Transformed	43.4	56.5	65.4	54.9	30.2
	Per cent	47.5	68.1	82.1	66.3	26.7

Necessary difference, 5% level, between controls and treatments 6.5, treatments 7.5 (transformed data).

Interaction effects of phytohormone and cane sugar dust treatments are shown in Table III. The data are averages for treatment with and without organic mercury and for both media. The combination of sugar with indolylacetic acid caused a reduction in the number of rooted cuttings at the 1000, but a slight increase at the 5000, p.p.m. concentration. Rooting tended to be reduced at 1000 p.p.m. naphthylacetic acid in the presence of sugar and this reduction reached significance at the 5000 p.p.m. level. It is apparent that the beneficial effects of 1000 p.p.m. naphthylacetic acid, given in Table II, were obtained in the absence of sugar.

Rooting was increased by the treatment with 50 p.p.m. of organic mercury from 48.8 to 54.5%—the figures being averages over all other treatments.

Number of Roots per Rooted Cutting

The average of the number of roots per rooted control cutting was 4.1. Phytohormone treatment increased this number by approximately one.

Indolylacetic acid was somewhat more effective than naphthylacetic acid, and the stimulatory effect of hormone treatment was more marked in sand-peat than in sand.

TABLE III

AVERAGE EFFECTS OF PHYTOHORMONE AND CANE SUGAR DUSTS ON THE NUMBER OF CUTTINGS ROOTED

Concentration of sugar, %	Data	Phytohormone concentration in talc, p.p.m.				
		0	Indolylacetic acid		Naphthylacetic acid	
			1000	5000	1000	5000
0	Transformed	43.9	53.2	59.0	50.8	31.0
	Per cent	48.1	62.9	72.5	59.2	27.9
10	Transformed	46.3	46.1	62.5	48.9	14.8
	Per cent	52.3	51.7	77.9	56.7	10.4

Necessary difference, 5% level, between controls 5.3, controls and treatments 6.5, treatments 7.5 (transformed data).

Total Root Length per Rooted Cutting

The average total root length per rooted cutting was about 8.5 cm. in sand and 13.0 cm. in sand-peat. No significant increase in total root length due to treatment was demonstrable in sand, but in sand-peat, the 15.7 cm. length observed on the rooted cuttings treated with 5000 p.p.m. indolylacetic acid was significantly greater than the value of 11 cm. for the controls. On the average, organic mercury treatment increased the length of root from 9.5 to 11.5 cm.

Mean Root Length

The mean root length was about 2.5 cm. in both media. Of the various chemical treatments, the 1000 p.p.m. concentration of naphthylacetic acid resulted in the greatest mean root length, 3.0 cm. in the presence of organic mercury and the absence of cane sugar, when averaged over both media. The statistical analysis also revealed, however, that whereas naphthylacetic acid treatment resulted in a greater mean root length in sand than indolylacetic acid treatment, in sand-peat mean root length was greater after application of indolylacetic acid.

Number of Surviving Cuttings

Observations in early August indicated an average survival of 92.5%. Despite this high average, the data demonstrated increased survival from indolylacetic acid treatments and the 1000 p.p.m. concentration of naphthylacetic acid. While sugar treatment increased survival of the controls without phytohormone, in combination with the 5000 p.p.m. concentration of naphthylacetic acid it was injurious. Similarly, organic mercury was beneficial in the absence of phytohormone but injurious with the highest concentration of naphthylacetic acid. For the most part, there was no significant difference

between media, but the injurious effects of 5000 p.p.m. naphthylacetic acid were reduced in sand-peat.

Average survival was reduced to 77.6% at the September count. The effects of both sugar and organic mercury treatments were essentially the same as in the earlier observations. The 5000 p.p.m. concentration of naphthylacetic acid resulted in survival of 50% in the absence, and 18% in the presence, of cane sugar. Organic mercury, similarly, accentuated the injurious effects of the highest concentration of naphthylacetic acid. However, in the absence of phytohormone, organic mercury increased survival from 76 to 84%. Survival after treatment with mercury in combination with both concentrations of indolylacetic acid and the 1000 p.p.m. concentration of naphthylacetic acid was not significantly greater than after organic mercury or phytohormone alone.

TABLE IV

AVERAGE EFFECTS OF PHYTOHORMONE DUST TREATMENTS AND MEDIA ON THE NUMBER OF CUTTINGS SURVIVING IN SEPTEMBER

Medium	Data	Phytohormone concentration in talc, p.p.m.				
		0	Indolylacetic acid		Naphthylacetic acid	
			1000	5000	1000	5000
Sand	Transformed	68.7	75.4	78.2	65.6	29.5
	Per cent	84.8	92.5	93.8	82.1	27.1
Sand-peat	Transformed	60.9	77.5	75.3	74.0	39.5
	Per cent	75.0	92.9	92.1	89.6	41.2

Necessary difference, 5% level, between controls and treatments 7.4, treatments 8.5 (transformed data).

Table IV indicates the effects of media and phytohormone dust treatments on the number of surviving cuttings in September. Data are averages for treatments with and without cane sugar and organic mercury. Whilst the 5000 p.p.m. concentration of indolylacetic acid was the only treatment to increase survival in sand, and all but the 5000 p.p.m. concentration of naphthylacetic acid increased survival in sand-peat, it requires to be noted that the survival of the controls was lower in the second medium. The injurious effect of the high naphthylacetic dosage was however less evident in sand-peat. Although indolylacetic acid treatments in both media resulted in survival of the order of 93%, comparison with the data of Table II indicates that a substantially greater percentage of the surviving cuttings rooted in sand-peat than in sand only.

Development of New Growth

New growth, which developed on only 54% of the cuttings, began after the general appearance of growth on the parent tree and continued throughout most of the summer.

Effects of treatments with phytohormones and cane sugar on the number of cuttings with terminal new growth are given in Table V, in which the data are averages for treatments with and without organic mercury and in both media. The average effect of sugar was a decrease in the number of cuttings with terminal new growth, and that of phytohormone treatment an increase with 1000 p.p.m. indolylacetic acid. and a decrease following the highest concentration of naphthylacetic acid.

TABLE V

AVERAGE EFFECTS OF PHYTOHORMONE AND CANE SUGAR DUST TREATMENTS ON THE NUMBER OF CUTTINGS WITH TERMINAL NEW GROWTH

Cane sugar concentration, %	Data	Phytohormone concentration in talc, p.p.m.					Means for sugar treatments
		0	Indolylacetic acid		Naphthylacetic acid		
			1000	5000	1000	5000	
0	Average of 30 cuttings	17.7	18.2	17.5	17.6	8.0	16.1
	Per cent	59.0	60.8	58.3	58.8	26.7	53.8
10	Average of 30 cuttings	15.2	18.8	16.4	15.0	3.6	14.0
	Per cent	50.6	62.5	54.6	50.0	12.1	46.7
Means for treatments	Average of 30 cuttings	16.4	18.5	16.9	16.3	5.8	
	Per cent	54.8	61.7	56.5	54.4	19.4	

Necessary difference, 5% level, between controls without phytohormone 2.4, controls and treatments 2.9, treatments 3.3, controls and treatment means 2.0, treatment means 2.4 (averages of 30).

The effect of media, averaged over all other treatments, was highly significant, 41.8% of the cuttings in sand against 58.7% of those in sand-peat developing terminal new growth.

Whereas 50% of the cuttings showed terminal growth, an additional 4% had lateral new growth without terminal. Inclusion of these latter had little effect on the treatment differences shown in Table V, but did result in the demonstration of a significant reduction in the total number of cuttings with new growth following treatment with both concentrations of naphthylacetic acid in combination with cane sugar.

Data for the effects of phytohormone and cane sugar dust treatments on the average number of new growth shoots per group of 30 cuttings are given in Table VI. These are averages for treatments with and without organic mercury and in both media. It is apparent that treatment effects were similar to those indicated in Table V. There was also a significant difference between media in this respect. In the mixture of peat and sand there were on the average 25.5 shoots per group, as compared to only 17.1 in sand. The beneficial effects of the mixture of sand-peat were also seen in the length of new growth produced. Random selection of 121 cuttings propagated in sand gave an average length of 2.2 cm. per shoot, while the average for 146

cuttings from sand-peat was 4.0 cm.. Thus, the addition of peat increased the length of growth as well as the number of shoots and the number of cuttings producing growth.

TABLE VI

AVERAGE EFFECTS OF PHYTOHORMONE AND CANE SUGAR DUST TREATMENTS ON THE NUMBER OF NEW GROWTH SHOOTS PER GROUP OF 30 CUTTINGS

Cane sugar concentration, %	Phytohormone concentration in talc, p.p.m.				
	0	Indolylacetic acid		Naphthylacetic acid	
		1000	5000	1000	5000
0	23.8	26.3	29.0	24.3	10.0
10	19.7	30.0	25.5	19.9	4.3

Necessary difference, 5% level, between controls without phytohormone 3.3, controls and treatments 4.0, treatments 4.6.

Number of Rooted Cuttings with New Growth

The effects of phytohormone and cane sugar treatments on the number of rooted cuttings with new growth were similar to those given in Table III. Of the total number of rooted cuttings, the proportion with new growth ranged from about 50% for the controls to 60% for the indolylacetic acid treatments. The treatment with 1000 p.p.m. naphthylacetic acid in the absence of sugar resulted in 61% of the rooted cuttings having new growth.

On the average, there was an increase in the number of rooted cuttings with new growth following treatment with organic mercury in the absence, but not in the presence, of cane sugar, but at 5000 p.p.m. of both hormones organic mercury was beneficial in the absence, but detrimental in the presence, of sugar. In conjunction with the 1000 p.p.m. concentration of both hormones, organic mercury treatment increased the number of rooted cuttings with new growth by about 5%, and the addition of sugar had no effect. The beneficial effect of organic mercury in the case of controls without phytohormone was greater in sand-peat than in sand in the absence of sugar. This difference between media disappeared in the presence of sugar. In conjunction with 5000 p.p.m. naphthylacetic acid, on the contrary, the effect of organic mercury was greater in sand than in sand-peat. This difference also vanished on the addition of sugar.

While only 19.4% of the total of cuttings planted were rooted and had new growth in sand, the corresponding percentage was 38.0 in sand-peat.

Incidence of New Growth in Relation to Subsequent Rooting

Study of the relation of media and phytohormone treatments to the effect of new growth on rooting revealed that the main hormone effect in sand was due to the 5000 p.p.m. concentration of naphthylacetic acid. After this treatment the presence of new growth favoured rooting, whereas both the

controls and other treatments were followed by better rooting of the cuttings without new growth. In sand-peat the 1000 p.p.m. concentration of indolylacetic acid favoured rooting of cuttings without new growth. This was the only significant effect. On the average, new growth was harmful to rooting in sand and had no effect in sand-peat. Over the entire experiment 12.3% more of the cuttings without new growth were rooted than of those with such growth.

Discussion

Outside propagation of this November collection of Norway spruce cuttings resulted in much better rooting than was obtained in the greenhouse with cuttings of the same collection, although it is true that the propagation media were not identical (9). While the earlier experiment indicated a beneficial effect from 1000 p.p.m. indolylacetic acid, which effected rooting of 42%, an increase of about 10% above the controls, the 5000 p.p.m. concentration and both concentrations of naphthylacetic acid either had no effect or were injurious. In the present experiment, however, all the phytohormone treatments but the 5000 p.p.m. naphthylacetic acid had a marked beneficial effect. It is clear therefore that the results of greenhouse experiments cannot in all cases be used readily to evaluate the effectiveness of phytohormone treatments under outside conditions.

The beneficial effects of the sand-peat medium were an outstanding feature of the results. In the main, these related to the interactions between phytohormone treatments and the medium. However, differences between media were marked in relation to effects on new growth. The substantial stimulation of new growth in sand-peat may be related to nutrient factors from the peat, since nutrient salts applied to a sand medium have been shown to affect new growth by the cuttings (6). It is particularly interesting to note that the combination of sand-peat with certain phytohormone treatments resulted in marked increase in the number of cuttings rooted and, at the same time, an increase in both the number and length of new growth shoots. High percentage rooting and vigourous top growth are of obvious importance in the development of spruce plantations by means of vegetative propagation.

The beneficial effects resulting from treatments with cane sugar and, more particularly, organic mercury are similar to those noted in earlier experiments (7, 9). However, for the most part, these effects were comparatively small and depended on interactions with phytohormone treatments, particularly the high concentration of naphthylacetic acid.

Greenhouse experiments have indicated that the best rooting is obtained with early winter collections of dormant material. The present November collection likewise gave much better rooting than was obtained on the outside propagation of an April collection of dormant cuttings (10).

It has been stated that the cuttings should preferably be moistened prior to dusting if appreciable effects are to be obtained from phytohormone treat-

ment (14). This conclusion appears rather too general since the cuttings in this experiment were not moistened prior to dry dusting.

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YIELDING CAPACITY OF WHEAT CROSSES AS INDICATED BY BULK HYBRID TESTS[1]

By J. B. Harrington[2]

Abstract

In order to determine readily the usefulness of heterosis as an indicator of the yielding capacity of cereal crosses, replicated half rod row yield trials were made with bulk unselected seed of ten wheat crosses in F_2 and of six in F_3. The practical yielding value of the latter six crosses was ascertained later by replicated rod row yield tests of selected lines in F_6, F_7 and F_8. All the F_2 populations (excepting a *T. vulgare* × *T. persicum* cross) yielded significantly more than the mean of the parental varieties, the increased yield being 52 and 64% for two of the crosses. Of the six F_3 populations tested, the only one to yield significantly above the parental average was Reward × Hope, and the only ones to yield significantly less than the parental average were the two having Quality as one parent. Replicated plot tests of purified lines selected for high yield and other agronomic virtues showed the Reward-Hope combination to be particularly valuable, whereas the two crosses containing Quality were unpromising. The results suggest that replicated bulk F_2 tests may be used to indicate the yielding potentialities of wheat crosses, and that bulk F_3 tests may have supplementary value in this regard.

The breeder of cereal crops makes many crosses, each with a definite purpose, but he has no sure way of knowing their comparative values in advance. This is particularly true respecting the very important attribute "yielding capacity".

It has been established that certain varieties have a higher value in combining ability than others. This is illustrated by recent work in barley and oats. Harlan, Martini, and Stevens (6) carried 379 barley crosses separately in unselected mass for seven years and recorded the yields of single 10-ft. rows. They found that some crosses yielded much more than others and that the average yield of selections from the higher yielding crosses decidedly surpassed the yields of selections from the lower yielding crosses. Coffman *et al.* (2) showed the oat variety Markton to be a better parent than several other varieties.

An outstanding advance in the development of tests designed to reveal superior combining ability is the development of the "top crossing" principle. Lindstrom (10) announced this principle in 1931 as a valuable technique in maize improvement. Jenkins (9) found that crosses of inbred lines of corn with a commercial variety (top crossing) could be used satisfactorily for testing the yielding value of the selfed lines as parents in single and double crosses.

Studies on the intricate yield mechanism of plants have been made, by Bridgeford and Hayes (1), Waldron (13), and many others, but with all this work the breeder was not furnished with a safe rapid method of predicting the probability of obtaining high yielding segregates from a given cross.

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An approach to the subject was made by the writer (7) in 1932 in a paper on predicting the value of a cross from an F_2 analysis. In that paper the writer concluded that the data on several hundred individual F_2 plants gave a reasonably accurate prediction of the yielding value of the wheat cross Marquillo × Marquis which was studied. This attack on the problem was satisfactory but it had two disadvantages, one being the cost of detailed analyses of hundreds of F_2 plants and the other the lack of direct comparison with other crosses.

Coffman and Wiebe (3), in an unreplicated rod row test in 1938, found that F_2 populations outyielded the average of the parent varieties in three out of four oat crosses studied. No attempt was made to interpret the results in terms of the relative yielding capacities of the crosses.

Since yield depends upon many genetic factors, it is reasonable to assume that the probability of obtaining high yielding segregates would be greater in a cross between parents with a larger total of yield genes than in one between parents possessing relatively few such genes. If this assumption is correct, heterosis, as shown by the extent to which the earliest hybrid generations excel the parental varieties in yield, should be a true indication of the yield value of a cross. In 1930 the writer began a study in which F_2 and F_3 bulk populations were given replicated plot tests to furnish information on the relation between heterosis and the yielding value of different varietal combinations in wheat. The F_1 was not used owing both to the cost of producing sufficient hybrid seed for adequate plot tests and to the difficulty of making satisfactory comparative yield tests of individual plants of the F_1 and the parental varieties.

Materials and Methods

Six crosses of compatible varieties differing in yielding ability were made in 1930 and the unselected bulk F_2 and F_3 generations of each cross were placed in replicated half-rod-row yield trials in 1932 and 1933 along with the parental varieties. The crosses* were as follows: Quality × Ceres, Reward × Ceres, Quality × Reward, Reward × Hope, Garnet × Reward and Reward × Reliance.

In order to confirm or disprove the conclusions drawn from the F_2 and F_3 tests with regard to the comparative yielding capacities of the different crosses, standard six replicate rod row trials were made on selections from the crosses as soon as reasonably true breeding lines were obtained. These

* *Following are the pedigrees of the varieties used in the crosses (S means Saskatchewan Accession Number, C means Canadian Accession Number): Apex (S1789, C1909) from H-44-24 (sib of Hope) × Double Cross (sib of Thatcher) × Marquis; Black Persian (S1801, C1238) introduction typical of T. persicum; Bobs (Sask. 1688, C1665) supposedly from a cross of Nepal barley with Early Lambrigg wheat; Ceres (S1212, C1263) Kota × Marquis; Garnet (S791, C1316) Riga (Ladoga × Red Fife) × Preston (Onega × Gehun); Hope (S1663, C1339) Vernal emmer × Marquis; Marquis (S100, C1691) Red Fife × Hard Red Calcutta; Mayview (S1286, C1694) a compactum selection from Fortyfold; Pioneer (S1472, C1467) sib of Garnet (Riga × Preston); Quality (S1200, C1490) probably sib of Florence (White Naples-Improved Fife × White Naples × Improved Fife-Eden); Reliance (S1851, C1498) Kanred (selection from Crimean) × Marquis; Reward (S1003, C1509) Marquis × Prelude (from Ladoga-White Fife × Hard Red Calcutta × Gehun); Thatcher (S1720, C1820) Kanred-Marquis × Iumillo durum × Marquis.*

tests were made on the lines from five of the crosses during the period 1936 to 1938. The sixth cross, Reward × Hope, was not continued at Saskatoon, largely on account of the extensive work with this combination of parents at another experiment station.

To furnish further information on the behaviour of different crosses a six replicate rod row plot test was made in 1939 of bulk F_2 of four crosses* ranging from a cross between closely related parents, Reliance × Thatcher, to a cross between Marquis and Black Persian *(T. persicum)* involving species of different chromosome number. The other crosses were Bobs × Pioneer, where the relationship is geographically wide, and Mayview *(T. compactum)* × Apex, a cross between related species of similar chromosome number.

The 1932, 1933 and 1939, tests were made according to the modified latin square of groups plan, each F_2 and its parental varieties constituting a group. The essentials of the method were described recently by Wishart (14). The plots were single rows eight feet long and one and a half feet apart. The number of replicates was four in 1932, three in 1933 and six in 1939.

The 1936, 1937 and 1938 tests were made according to the modified latin square plan. Triple rod row plots with rows one foot apart were used.

While a full record of plant height, days to heading, days to maturity, grain size, grain plumpness and several other agronomic attributes was obtained, it was considered that this paper could well be limited to a consideration of yield.

Results

The results from the six original crosses are presented in Table I. The F_2 of each of the crosses yielded significantly above the average of the parents. In four crosses the F_2 yielded significantly better than the higher yielding parent.

The F_3 yielded quite differently than the F_2. Only Reward × Hope F_3 significantly outyielded the parental variety average. The F_3 of both crosses involving Quality yielded significantly less than the parental variety averages.

The yield tests of selected purified lines in F_6, F_7 and F_8 showed no significant difference between parental variety average and line average in any cross. However, lines yielding as well as the higher yielding parent were obtained from Reward × Ceres, Garnet × Reward and Reward × Reliance. The Reward × Hope cross was discarded after the F_6 test, but lines significantly excelling both parental varieties in yield were obtained from this cross at the Dominion Experimental Farm, Brandon, Canada (11). Lines superior in yielding capacity were not obtained from the Quality × Ceres or Quality × Reward cross.

The results from the 1939 bulk F_2 yield test are given in Table II. The four crosses are arranged in order of estimated closeness of genetic relationship. The Thatcher × Reliance F_2 was significantly excelled by Reliance. The F_2 of Bobs × Pioneer, on the other hand, greatly excelled both parents. The same is true of the F_2 of Mayview × Apex only the contrast is more startling,

the F_2 yielding more than twice as much as Mayview. The Marquis × Black Persian cross gave a very poor F_3 yield owing to the high sterility of most of the segregates.

TABLE I

YIELDS FROM REPLICATED HALF ROD ROW TESTS OF WHEAT CROSSES IN UNSELECTED BULKED F_2 AND F_3 AND IN SELECTED LINES CARRIED IN REPLICATED ROD ROW TESTS IN F_6, F_7 AND F_8

Mean plot yld.	Quality × Ceres‡		Reward × Ceres‡		Quality‡ × Reward		Reward‡ × Hope		Garnet‡ × Reward		Reward × Reliance‡	
	Gm.	%	Gm.	%	Gm.	%	Gm.	%	Gm.	%	Gm.	%
F_2 bulk 1932	187	109	199	117	173	119	201	152	200	125	203	121
Low parent	147	85	143	84	143	99	121	92	143	90	143	85
High parent	197	116	197	116	147	101	143	108	176	110	193	115
Avg. parent	172	100	170	100	145	100	132	100	160	100	168	100
F_2-Avg. par.	15	9	29	17	28	19	69	52	40	25	35	21
Nec. diff.*	10		10		9		9		10		10	
F_3 bulk 1933	50	78	69	104	49	75	74	112	68	100	74	104
Low parent	63	98	67	102	67	103	64	97	67	98	67	94
High parent	64	100	64	97	63	97	67	102	68	100	75	106
Avg. parent	64	100	66	100	65	100	66	100	68	100	71	100
F_3-Avg. par.	−14	22	3	5	−16	25	8	12	0	0	3	4
Nec. diff.*	4		5		4		5		5		5	
Mean acre yld.	Bu.	%	Bu.	%	Bu.	%	Bu.	%	Bu.	%	Bu.	%
F_6 lines §1936			19.6	95					13.9	109	15.2	107
Low parent			22.6	110					12.5	98	14.5	102
High parent			18.5	90					13.0	102	13.8	97
Avg. parent			20.6	100					12.8	100	14.2	100
F_6-Avg. par.			−1.0	5					1.1	9	1.0	7
Nec. diff.*			3.9						2.0		1.4	
F_7 lines 1937	4.3	96	5.5	95	4.2	76			8.6x	92	3.2†	84
Low parent	4.9	109	6.7	116	5.8	106			9.7x	104	4.7	124
High parent	4.0	90	4.9	84	5.2	94			9.0x	97	3.0†	79
Avg. parent	4.5	100	5.8	100	5.5	100			9.3	100	3.8	100
F_7-Avg. par.	−0.2	4	−0.3	5	−1.3	24			−0.7	8	−0.6	16
Nec. diff.*	1.2		1.8		1.5				1.3		0.9	
F_8 best 2 lines			22.5	121					24.5	128	20.4	117
F_8 lines 1938			20.7	111					22.2	116	18.7	108
Low parent			17.4	94					17.4	91	17.4	100
High parent			19.8	106					21.0	109	17.3	99
Avg. parent			18.6	100					19.2	100	17.4	100
F_8-Avg. par.			2.1	11					3.2	17	1.3	8
Nec. diff.*			4.4						4.6		3.1	

NOTE: *The F_6 of Quality × Ceres and Quality × Reward was not tested until 1937.* ‡*The higher yielding parental variety in 1932.* **Between hybrids and average of parents.* †*Later maturity greatly depressed the yields.* x*Extreme earliness considerably increased the yields.* §*The yields for lines are each averages of all lines tested. The numbers of lines averaged are as follows: Quality × Ceres 22 in F_6; Reward × Ceres 22 in F_6, 15 in F_7, 11 in F_8; Quality × Reward 8 in F_6; Reward × Hope 9 in F_6; Garnet × Reward 18 in F_6, 9 in F_7, 5 in F_8; Reward × Rel. 45 in F_6, 36 in F_7, 19 in F_8.*

TABLE II

REPLICATED ROD ROW TEST OF BULK F_2 AND PARENTAL VARIETIES OF FOUR CROSSES IN 1939

Variety or Hybrid	Sask. Acc. No.	Grain Yield in Bushels per Acre
Thatcher × Reliance F_2		43.9 ± 3.0
Reliance	1851	53.2 ± 3.7
Thatcher	1720	44.0 ± 3.0
Bobs × Pioneer F_2		49.8 ± 3.4
Bobs	1275	40.9 ± 2.8
Pioneer	1472	36.5 ± 2.5
Mayview × Apex F_2		54.9 ± 3.8
Apex	1789	41.7 ± 2.9
Mayview	1286	25.4 ± 1.8
Marquis × Black Persian F_2		11.2 ± 0.8
Marquis	100	47.3 ± 3.3
Black Persian	1801	38.0 ± 2.6

Source of variation	Deg. of freedom	Sum of squares	Mean squares	F value	5% point	1% point
Total	71	3060399	43104			
Between replicates	5	70182	14036	1.10	2.90	4.56
Between groups	3	496508	165503	13.00	3.29	5.42
Error (a)	15	190912	12728			
Between varieties	8	1853341	231668	20.62	2.18	2.99
Remainder, Error (b)	40	449456	11236			

Mean = 40.6 bu.

Within groups SE_v = 2.8 bu. Nec. diff. = 7.0 bu. SE_v = 6.9%.

Between groups SE_v = 2.8 bu. Nec. diff. = 7.1 bu. SE_v = 7.0%.

Discussion

It might be argued that heterosis as an indicator of yielding capacity would be unreliable since the dominance of genes for low yield might result in little or no heterosis, and crosses carrying such genes would be discarded as unpromising although they might have valuable recessive genes for high yielding capacity. While such a situation is possible, the writer finds no evidence to support it. Recently Hutchinson (8) stated, in a paper on cotton crosses, that the dominance bias is in all cases in the direction in which recent selection has operated. If we accept this statement as having a wide application it would follow that most of the genes associated with high yielding capacity tend to be dominant, and that therefore the amount of heterosis as displayed by a hybrid population should be indicative of the total inherent yielding capacity of the hybrids. However, East (4), in presenting the theory of linked growth factors as being the most reasonable explanation of hybrid vigour, states that "universal experience is to find 'dominance', in the classical sense,

virtually absent". Apparently as far as the plant breeder is concerned the "dominance" concept does not need to be considered.

The complexity of heterosis, with its dependence on linkage, and the value of many characters other than those associated with growth make it difficult to provide from parental performance or in F_1, F_2 or F_3 a suitable method of estimating the probable value of a cross. Heterosis in a naturally self fertilized crop such as wheat is usually striking in F_1 and moderate in F_2. But some varietal combinations show no heterosis, and in others hybrid vigour may appear for some characters and not for others, as found by Rosenquist (12) in his study of hybrid vigour in wheat. The vigour importance of such characters as stooling propensity, height of plant, head length, seed size, and length of growth period varies with the material used. But, as East (4) points out, hybrid vigour is a phenomenon affecting the plant as a whole. And, as yield is a result of the operation of the plant as a whole, most investigators agree that increase in yield is a reliable index of the extent of heterosis.

The results in this paper, as well as in many other studies, indicate the dependence of heterosis upon the genetic differences of the parental stocks. It seems reasonable to expect a larger proportion of high yielding segregates from crosses involving parents with a high degree of genetic disparity than from crosses with more closely related parents. Therefore, it seems fair to assume that the amount of heterosis indicates the total inherent yielding capacity of a cross.

Considering the F_2 and F_3 results presented in Table I from this point of view, one would expect the Reward × Hope cross to be the most promising and the two crosses involving Quality to be the least promising. Actual results fully justify this prediction. The Hope-Reward combination produced many high yielding lines, whereas Quality × Ceres and Quality × Reward were so unpromising they were discarded after the F_6 tests.

If the results in Table II are interpreted in the same manner, it must be concluded, as far as yield is concerned, that there is no particular value in carrying on the cross Reliance × Thatcher. It must also be concluded that the crosses Bobs × Pioneer and Mayview × Apex have excellent yield possibilities.

While the results in this paper are from relatively few crosses they suggest that bulk F_2 yield trials may be used to indicate the potential yield possiblities of a cross. In addition the results in Table I suggest that F_3 yield trials are of supplementary value as a support to the F_2 conclusions.

Nevertheless, regardless of the usefulness of bulk hybrid tests in evaluating the yield potentialities of crosses, a number of economic characters other than yield may be limiting factors. Milling and baking quality, disease resistance, and resistance to certain weather conditions are some of these characters. For such characters the bulk hybrid trials may be of little use. For example, the cross Reliance × Thatcher (see Table II) involves two varieties of very high yielding capacity. The purpose of the cross is to combine the ability

of Reliance to produce a large, plump, heavy kernel with Thatcher's ability to resist stem rust. A bulk, F_3 test of this cross might well be considered of questionable value.

While the present study has furnished information of value on the usefulness of bulk hybrid trials in assaying the values of crosses, the writer feels that there is need for more extensive studies along the same line, not only in wheat but in other crops and that larger numbers of crosses should be involved in such studies. It would also appear to be worthwhile to have parental varieties F_1, F_2, and F_3 in the same replicated yield trial as suggested in 1932 by Fisher, Immer, and Tedin (5). Experiments along these lines are now under way on barley and wheat at a number of places, including Saskatoon.

Acknowledgments

The writer acknowledges with much appreciation the assistance of all those who helped with the investigation, especially Mr. H. A. Purdy who made the F_2 and F_3 tests in 1932 and 1933, and Mr. J. Whitehouse who made the F_3 test in 1939 and performed the variance analysis of the data.

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WINTER GROWTH IN THE VEGETATIVE BUDS OF THE WAGENER APPLE[1]

By Hugh P. Bell[2]

Abstract

Vegetative buds of the apple were collected from September 26, 1938, to March 18, 1939. The median longitudinal section of each bud was measured. The data collected suggested that *a slow but continuous growth in length* within the bud proceeds throughout the winter months. The figures were subjected to statistical analysis and found to be significant.

Introduction

A study of the vegetative bud of the apple throughout a number of winters, suggested that a slight increase in axis length took place even during the coldest months (2, p. 348). The work reported below was undertaken to obtain more detailed and accurate information on this subject.

The literature is not very helpful. There are many references to the vital activities during dormancy of seeds, but very few to dormancy of vegetative buds. In fact, no recent article could be located that gave definite information regarding growth in size within the vegetative bud during the winter. The standard textbooks on plant physiology are usually noncommittal on the subject, but some make definite statements and these are not always in agreement; for instance, Jost (4, p. 345) states: "According to Askenasy's (1877) researches a *complete rest*, where growth is at an absolute standstill, does not take place in the buds. In the flower-buds of the cherry a continuous but feeble *embryonic* growth goes on from summer till next spring." Askenasy (1, p. 804) studied the flower-buds of the cherry and what he actually says is: "The development of the flower-buds of the cherry is divided into two periods which are separated by a period of rest or very small growth." In apparent disagreement with Jost's statement, Maximov (6, p. 290) deals with winter activity within the bud as follows: "In general, all vital activities manifest themselves during dormancy with the exception of growth, which does not take place, though the external conditions may be quite favourable for it." In view of the above, a short statement regarding the data obtained on this subject should be of value.

[1] *Manuscript received July 25, 1940.*
Contribution from the Department of Biology, Dalhousie University, Halifax, N.S., with financial assistance from the National Research Council of Canada.

[2] *Professor of Botany.*

Materials and Methods

The material for this investigation was obtained from a normal Wagener tree at the Dominion Experimental Farm, Kentville, Nova Scotia. Buds, collected twice each month from September 26, 1938, to March 18, 1939, were killed in chrom-acetic. In the selection of individual buds great care was taken to make each one a "random selection", and moreover each collection included buds from all sides and parts of the tree and from different parts of the branch. This material should be typical for winter buds in Nova Scotia, for the meteorological reports indicate that the winter of 1938–39 was an average season. As an example, the winter temperature records from September, 1935, to March, 1940, are given in Table I.

TABLE I

WINTER TEMPERATURES AT KENTVILLE, NOVA SCOTIA, DEGREES F. SEPTEMBER, 1935 TO MARCH, 1940

	Max.	Min.	Mean
Sept. 1935	83	33	55.81
Oct. 1935	73	24	47.10
Nov. 1935	68	23	42.22
Dec. 1935	43	−9	25.49
Jan. 1936	50	−5	22.29
Feb. 1936	47	−4	17.54
Mar. 1936	68	4	38.79
Sept. 1936	78	32	56.75
Oct. 1936	73	21	47.18
Nov. 1936	68	7	35.5
Dec. 1936	57	0	27.76
Jan. 1937	55	0	26.37
Feb. 1937	46	−8	25.6
Mar. 1937	46	8	28.4
Sept. 1937	89	34	59.04
Oct. 1937	71	26	47.4
Nov. 1937	59	13	38.17
Dec. 1937	50	−7	26.87
Jan. 1938	57	−13	21.26
Feb. 1938	42	−11	17.73
Mar. 1938	57	−15	25.52
Sept. 1938	82	33	57.80
Oct. 1938	78	27	49.34
Nov. 1938	70	11	39.78
Dec. 1938	59	2	28.57
Jan. 1939	45	1	21.02
Feb. 1939	58	−7	21.54
Mar. 1939	50	1	24.57
Sept. 1939	89	29.5	58.56
Oct. 1939	78	20	48.94
Nov. 1939	56	16	34.22
Dec. 1939	59	7	27.20
Jan. 1940	35	−6	16.12
Feb. 1940	46	−10	20.67
Mar. 1940	57	−5	28.57

It is difficult to estimate growth in length within a bud, for when preparing material for imbedding in paraffin, it is necessary to remove the outer scales and young leaves. Hence there is no "bench mark" or "base line" from which to make measurements. To overcome this difficulty, the buds were not dissected, and the basal portion of the petiole of the most distal leaf was left attached. The buds were then imbedded in celloidin and cut so that the median longitudinal section passed through the base of this most distal petiole. This median section was projected on drawing paper at a magnification of 53 and the outline was sketched. On each sketch a line was drawn from the centre of the crown down through the central axis of the bud, and a perpendicular was dropped on this line from the axil of the leaf, that is, from the point where the ventral surface of the petiole joined the stem just below the terminal bud. The distance along the median line from the centre of the crown to the point of juncture with the perpendicular, was taken as the *height of the bud axis*, and the distance along the perpendicular from the axil of the leaf to the central axis line, was taken as the *radius of the bud base*. These two dimensions were measured on the outline of each median section.

Results and Discussion

Owing to the great variation in size of the buds in each collection, it appeared that the most reasonable way to test for increase in height of the bud axis was to study the ratio of height to radius. A typical example of the measurements for one collection is given in Table II. The averages of the measurements and their ratios, for each collection, are given in Table III. A graphic representation of the ratios is given in Fig. 1. It will be seen that there is some indication of an increase in height during the winter months, provided that it can be assumed that the radius for an individual bud does not change materially during that same period. The measurements obtained (Table III) appear to justify this assumption.

Mr. John S. Leefe of the Dominion Experimental Farm, Kentville, N.S., and Dr. Donald Mainland, Professor of Anatomy, Dalhousie University,

TABLE II

MEASUREMENTS OF THE BUDS COLLECTED ON OCTOBER 3, 1938. THE ACTUAL SIZE OF THE BUD IS MAGNIFIED BY 53

Bud No.	Height of bud axis, cm.	Radius of bud base, cm.	Ratio of height of bud axis to radius of bud base
1	7.60	6.95	1.09
2	7.15	6.65	1.07
3	7.40	7.15	1.03
4	6.45	5.75	1.12
5	8.05	7.20	1.11
6	7.56	7.77	0.97
7	9.56	6.46	1.48
8	6.90	7.20	0.95
9	6.25	6.60	0.94
10	7.40	7.25	1.02
Averages	7.43	6.79	1.07

TABLE III

THE AVERAGE OF MEASUREMENTS AND THE RATIO FOR EACH COLLECTION. THE ACTUAL SIZE IS MAGNIFIED BY 53

Date of collection	Average height of bud axis, cm.	Average radius of bud base, cm.	Average of ratios of height of bud axis to radius of bud base	No. buds measured
26-9-38	5.68	6.01	0.94	9
3-10-38	7.43	6.79	1.07	10
17-10-38	6.86	6.47	1.05	10
31-10-38	6.87	6.20	1.07	11
14-11-38	8.08	6.94	1.16	10
28-11-38	7.32	6.34	1.15	11
12-12-38	7.02	6.42	1.08	10
27-12-38	6.44	5.95	1.08	11
9-1-39	7.37	6.63	1.11	10
24-1-39	6.74	5.63	1.07	9
6-2-39	8.10	6.80	1.19	11
24-2-39	7.22	6.16	1.17	11
6-3-39	7.05	6.31	1.16	10
18-3-39	7.61	6.28	1.30	11

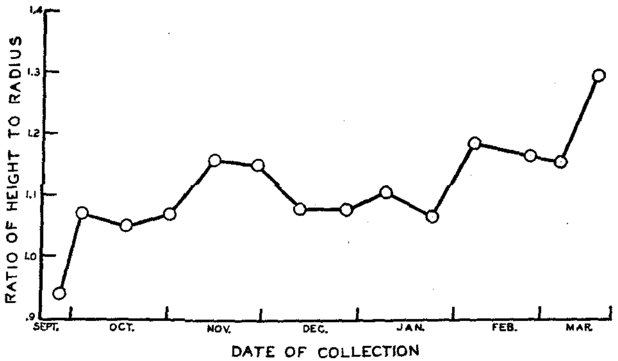

FIG. 1. *Variation in the ratio of height of bud axis to radius of bud base during the period of investigation. (Table III, Columns 1 and 4.)*

Halifax, N.S., have been kind enough to submit the data illustrated in Fig. 1 to statistical analysis, and the writer is indebted to Dr. Mainland for the following statement:

"Since no claim to generalization is based on this series of observations from a single tree in one winter, it does not seem desirable to give many numerical details of the statistical tests to which the data were subjected,

but rather to mention the methods, those prescribed by Fisher, (3), and the results of the tests.

"The data had been prepared as ratios of bud height to bud radius; but ratios are notoriously liable to introduce fallacies (5, p. 269), and in this instance a narrowing of the buds, i.e., a diminution of radius, would produce an increase in the ratio without increase in height. This possibility may not be botanically likely, but it was desirable to prove its absence. As would be expected, the taller buds had, on the average, the longer radii (coefficient of correlation (+0.59). There was no tendency for the radii to change within the period surveyed (correlation coefficient, −0.07), but the method of partial regression showed that, for any given radius, there was a statistically significant upward trend in the corresponding bud height. The partial correlation coefficient representing this relationship between height and time, with radius eliminated, was +0.26, and the regression coefficient, representing rate of increase in height, was 0.069 cm. per unit of time (about two weeks) in terms of the magnified bud sizes, i.e., actually about 13 μ.

"From the original height : radius ratios, a linear regression equation was derived, to represent the straight line that best fitted the graph in Fig. 1. Analysis of variance was carried out by the method of Fisher (3, sect. 44), the general principles of which are briefly described by Mainland (5, p. 280), and the following facts were demonstrated:

"(1) The slope of the regression line was significant, i.e., the increase in the ratios, represented by Fig. 1, could have occurred by chance less than once in 1000 times.

"(2) The increase in the ratio with lapse of time was adequately represented by the straight regression line, i.e., there was no reason to suppose that the downward slopes in the line of the ratios in Fig. 1 represent a real cessation of the main upward trend.

"(3) The average increase in the ratio per unit time interval of about two weeks was 0.0128.

"As just stated, there is no adequate reason to believe that the upward trend in bud size was other than uniform, i.e., the irregularities in Fig. 1 are not significant, but the graph suggests that future investigations be directed to the central period, namely the months of December and January."

In view of Dr. Mainland's statement, it can be concluded that there is a slow growth in the vegetative bud of the apple throughout the winter months, and that this growth produces an increase in length of the bud axis. About the December-January irregularity in the curve of Fig. 1 nothing can be stated with certainty until more observations have been made.

Acknowledgment

The author wishes to express his indebtedness to the staff of the Laboratory of Plant Pathology, Kentville, Nova Scotia, for making the collections of the buds during the winter of 1938–39.

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VEGETATIVE PROPAGATION OF CONIFERS

VIII. EFFECTS OF MEDIA AND PHYTOHORMONE DUST TREATMENTS ON THE ROOTING OF NORWAY SPRUCE CUTTINGS[1]

By N. H. Grace[2] and J. L. Farrar[3]

Abstract

Dormant Norway spruce cuttings collected in November were treated with talc dusts containing indolylacetic acid, planted in five media including two different sands and mixtures of these with peat humus, and propagated in a greenhouse. While 1000 p.p.m. indolylacetic acid treatment increased survival in sand and there were interactions between media and phytohormone treatments, the marked differences between the various media were the striking feature of the results. Mixtures of peat humus with sand were uniformly superior to sand only. There were also some differences between a fine and a coarse sand, when used either alone or in combination with peat. It may accordingly be concluded that selection of a suitable medium is of great importance in the propagation of Norway spruce cuttings.

It has been shown that the rooting response of Norway spruce cuttings depends on a number of factors (2–12). The two most important considerations observed to date relate to the stage of growth at which the cuttings are taken and to the media in which propagation occurs. While treatment with root growth stimulating chemical has resulted frequently in injurious effects, when these chemicals are applied by the carrier dust method some marked beneficial effects have been noted (7, 9). The most impressive results from use of these chemicals were obtained on treatment of dormant cuttings which were subsequently propagated in a mixture of sand and peat in equal proportions (9). Outside propagation in such a medium gave results superior to those obtained in sand only. The combination of certain media and chemical treatments had marked stimulatory effects on new growth as well as on root development. In view of these findings a greenhouse experiment has been carried out with various dust treatments used in conjunction with a series of five media. The results of this experiment are outlined in this communication.

Experimental

Norway spruce branches were collected November 8, 1939, from the lower region of the tree. The plantation was 19 years of age and situated at the Petawawa Forest Experiment Station, Chalk River, Ontario. In order to ensure a supply of cuttings representative of the entire plantation, not more than two branches were taken from any one tree. The branches were stored outside, covered loosely with straw, and cuttings prepared December 29.

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The cuttings ranged in length from 6 to not more than 12 cm. and were plain, without a heel of old wood. There were from five to six lateral cuttings to one terminal.

The experiment was carried out in a propagation frame which contained 60 glazed earthenware crocks, each provided with drainage. This experimental arrangement has been described in an earlier communication (5). The chief advantage of this arrangement was the isolation of the cuttings in each of the 60 crocks, preventing any contamination from cross diffusion and permitting the use of a number of different media. The propagation frame was provided with a factory cotton screen to reduce light intensity and maintain the humidity, and was situated in a greenhouse room whose temperature approximated 65° F. for the duration of the experiment.

The experiment was of factorial design and comprised four dust and five media treatments, giving 20 different combinations or treatment groups. The four dust treatments involved groups of 15 cuttings without treatment, with talc only, and with 1000 and 3000 p.p.m. (parts of chemical by weight to 1,000,000 parts of the talc mixture) of indolylacetic acid in talc. These four treatment groups were planted in fine sand, coarse sand, one-half of fine sand and one-half peat humus, one-half coarse sand and one-half peat humus, and, finally, three-quarters of coarse sand with one-quarter of peat humus (9). There were three replicate blocks of the 20 treatments. Within each block there were five replicates of the dust or phytohormone treatments, and four replicates of the media treatments. Each of the 60 crocks contained one group of 15 cuttings. The entire experiment required 900 cuttings.

TABLE I

SCREEN ANALYSIS OF SANDS USED AS MEDIA

Mesh, openings to the inch	Fine sand		Coarse sand	
	Retained, %	Passed, %	Retained, %	Passed, %
10	2.1	97.9	6.8	93.2
14	4.2	95.8	14.7	85.3
28	35.5	64.5	51.3	48.7
35	72.3	27.7	73.4	26.6
48	92.1	7.9	88.6	11.4
65	97.1	3.0	94.2	5.8
100	99.1	0.9	97.1	2.9
200	>99.9	<0.1	99.3	0.7

Brown builder's sand was obtained from two local pits and one sand apparently was considerably finer than the other. The results of screen analyses of these two sands are given in Table I. It is apparent that the "coarse" sand has more of the very large and very small fractions than the "fine" sand. The material designated as coarse sand actually forms a denser mass and has approximately 10% less pore space than the fine.

Cuttings were dusted and planted to a depth of about one inch as soon as possible, in no case more than two hours after preparation. It might be pointed out that the cuttings were not moistened prior to dusting, though they were kept covered with a moist cloth for the short period between preparation and dusting. Ottawa tap water was used for watering throughout the course of the experiment.

Fifteen weeks after planting, the cuttings were removed for examination. Record was made of the number of cuttings surviving, rooted, callused, with new growth, and with new growth and rooted. The number and length of roots were determined and from the resulting data were calculated the number and length of roots per rooted cutting and the mean root length. The number and lengths of all new growth shoots were determined. These data permitted calculation of the length of new growth per cutting with new growth, and length of terminal new growth per cutting with terminal new growth. Data on numbers of cuttings were subjected to the inverse sine transformation prior to analysis of variance (1). All the data were subjected to analyses of variance.

Living non-rooted cuttings were replanted in the respective media after the first examination and taken up for a final count eight weeks later.

Results

In Table II are given results of the analyses of variance. Part A of the table deals with the number of surviving cuttings and refers to the results in all five media. Owing to exceedingly poor rooting in sand with meagre data not suited to the analysis of variance procedure, analyses of the other observations were restricted to the three media containing peat-sand. These results are given in Part B of the table. Significant effects from media or chemical treatment were to be noted in 8 of the 10 sets of observations considered. In all eight the effect of media was significant—with one exception, highly significant. There were significant interactions between media and chemical treatments with respect to the number of surviving cuttings, the mean length of all new growth shoots and the length of terminal new growth.

Data for the effects of media and dust treatments on the number of surviving cuttings are given in Table III. The effects of media, averaged over all dust treatments, were such that all peat mixtures showed a greater number of surviving cuttings than sand only. Fine sand was below coarse in respect to number of surviving cuttings, but the mixture of peat with fine sand was superior to its combination with the coarse. The results indicated that the combination of one-quarter peat with three-quarters sand was not so good a medium as equal proportions of peat and sand. The interaction of media with phytohormone treatments was such that whereas the 1000 p.p.m. concentration of indolylacetic acid increased survival in both sand media, the higher concentration indicated a falling off in the beneficial effect of the chemical; these effects, however, were not observed in the peat-sand mixtures, which gave high survival rates irrespective of treatment. Talc alone suggested some beneficial effect in sand but statistical significance was not obtained.

TABLE II

Analysis of variance of responses to media and phytohormone dust treatments of Norway spruce cuttings

Source of variance	Degrees of freedom (A)—surviving cuttings only	Mean square—number of surviving cuttings	Degrees of freedom (B)—observations in three media with peat	Mean square								
				Number of rooted and callused cuttings	number of rooted cuttings	Number of roots per rooted cutting	Length of root per rooted cutting	Mean root length	Number of cuttings with new growth	Number of rooted cuttings with new growth	Mean length of all new growth shoots	Mean length of terminal new growth shoots
Replicates	2	187.2	2	256.2*	98.5	0.03	141.5	12.6	357.4	128.2	8.78	9.37
Treatments	3	143.3	3	159.1	146.4	0.40	151.9	24.1	63.1	88.2	1.66	8.19
Media	4	3097.4***	2	168.5	843.3***	0.61	3996.9**	351.0*	1491.1***	1034.4***	245.20***	459.70***
Interaction treatments × media	12	159.8*	6	73.2	81.5	0.12	605.1	92.6	59.7	49.1	20.16*	15.77*
Error	38	73.7	22	67.7	69.2	0.28	537.3	64.5	134.9	75.2	7.57	5.51

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

TABLE III

EFFECTS OF MEDIA AND PHYTOHORMONE DUST TREATMENTS ON THE NUMBER OF SURVIVING CUTTINGS

Treatment	Kind of data	Medium				
		Sand		Peat humus with		
		Fine	Coarse	One-half fine sand	One-half coarse sand	Three-quarters coarse sand
Untreated	Transformed	27.1	35.0	69.6	74.0	62.2
	Per cent	22.2	33.3	86.7	88.9	77.8
Talc	Transformed	36.5	48.2	70.8	77.9	60.9
	Per cent	35.6	55.6	84.5	93.3	75.6
1000 p.p.m. indolylacetic acid in talc	Transformed	49.5	56.3	67.5	62.7	65.8
	Per cent	57.8	68.9	84.5	77.8	82.2
3000 p.p.m. indolylacetic acid in talc	Transformed	26.3	43.1	75.7	66.9	65.1
	Per cent	20.0	46.7	91.1	84.5	82.2
Means, media treatments	Transformed	34.9	45.7	78.4	70.4	63.5

Necessary difference, 5% level between means of media treatments, 7.2; for interaction, 14.4.

TABLE IV

AVERAGE EFFECTS OF MIXTURES OF PEAT AND SAND ON THE RESPONSES OF NORWAY SPRUCE CUTTINGS

Response	Peat humus with			Necessary difference, 5% level
	One-half fine sand	One-half coarse sand	Three-quarters coarse sand	
Number of rooted cuttings, transformed data	44.0	37.5	27.4	7.0
Per cent	48.3	37.2	23.3	
Number of roots per rooted cutting	2.7	2.6	2.4	
Length of roots per rooted cutting, mm.	64.4	39.6	28.9	19.6
Mean root length, mm.	22.8	15.2	12.2	8.1
Number of cuttings with new growth, transformed data	64.1	57.1	42.3	9.8
Per cent	78.9	68.9	45.6	
Number of rooted cuttings with new growth, transformed data	41.7	35.1	23.4	7.3
Per cent	44.4	33.3	17.8	
Mean length of all new growth shoots, mm.	25.3	19.5	16.3	2.3
Mean length of terminal new growth shoots, mm.	28.1	21.0	15.8	2.0

In Table IV are given data for the average effects of mixture of sand with peat humus on various other responses of the cuttings. Although several of the observed differences are statistically insignificant, it would seem that the mixture of equal proportions of peat with fine sand is to be preferred to the corresponding mixture with coarse sand. The poorest results were obtained with the medium low in peat. In several instances the differences ascribable to media were large in magnitude. Several of the responses indicated somewhat greater differences ascribable to the two proportions of peat rather than to the texture of the sand. A notable exception to this tendency was indicated by the length of roots per rooted cutting, substantially greater root length occurring in the fine sand. Although the mixtures of peat with the two sands did not differ significantly in respect to the number of cuttings with new growth, the combination with fine sand was much the best in so far as the mean length of all new growth shoots was concerned. However, the data on lengths of new growth shoots should be considered in detail in relation to the various dust treatments, as a significant interaction of these factors has been indicated in Table II.

TABLE V

MEAN LENGTH OF ALL NEW GROWTH SHOOTS, MM.

Medium	*Untreated* cuttings	*Cuttings treated with talc* containing indolylacetic acid, p.p.m.		
		0	1000	3000
50% peat humus, 50% fine sand	26.7	23.7	22.7	28.0
50% peat humus, 50% coarse sand	17.3	19.7	19.7	21.3
25% peat humus, 75% coarse sand	17.7	17.7	17.0	13.0

Necessary difference, 5% level, 4.6 mm.

Interaction effects of phytohormone dust treatments and media are indicated in the data of Table V, for the mean length of new growth shoots. In the mixture of peat and fine sand none of the treatments resulted in a mean length of new growth significantly different from that produced by the untreated cuttings. In the mixture of equal proportions of peat and coarse sand, shoot length tended to increase with phytohormone dosage, whereas in the mixture of 75% coarse sand and 25% peat this tendency was significantly reversed.

Similar data for the mean length of terminal new growth shoots are given in Table VI. The effects of phytohormone treatment were essentially the same as just described for mean shoot length in Table V. However, in the mixture of coarse sand and one-quarter peat, talc only resulted in a length of terminal new growth significantly greater than that shown by the untreated cuttings. Further, the highest level of indolylacetic acid did not reduce the length of terminal growth in any of the media.

TABLE VI

MEAN LENGTH OF TERMINAL NEW GROWTH SHOOTS, MM.

Medium	Untreated cuttings	Cuttings treated with talc containing indolylacetic acid, p.p.m.		
		0	1000	3000
50% peat humus, 50% fine sand	28.7	25.3	26.7	31.7
50% peat humus, 50% coarse sand	20.0	20.0	21.0	23.0
25% peat humus, 75% coarse sand	14.0	18.0	16.7	14.3

Necessary difference, 5% level, 3.9 mm.

Whereas the first examination had indicated that 36.3% of the total number of cuttings planted had rooted, the final count eight weeks later raised this to 43.5%. Thus, during the eight weeks subsequent to the first count an additional 7.2% of the cuttings rooted. However, statistical analysis of these final counts still failed to demonstrate any effects of chemical treatment on the number surviving. Media effects were again of the same order as at the first examination.

Discussion

The effects of media on the rooting responses of Norway spruce cuttings are the outstanding feature of these results. While meagre rooting in the two sands rendered consideration of complete data for this part of the experiment impossible, the results in the three peat mixtures indicate that under the conditions of this trial, the composition of the media had a much greater effect than had phytohormone dust treatments. The number of surviving cuttings was greater in coarse than in fine sand. However, in combination with peat the fine sand effected uniformly superior results. Differences attributable to the sand were most marked in measurements on root length and new growth. The amount of peat in the mixture appeared to have greater effect than the kind of sand on the number of rooted cuttings and the number rooted with new growth. These media differences would appear to be related to such factors as aeration, water holding capacity, or available essential chemical elements (5, 8, 9).

Although there were several interactions between dust treatments and media, phytohormone treatments failed to have marked effects.

These effects from mixtures of peat and sand are in general agreement with the results from the outside propagation of a November collection of cuttings (9), although the latter also indicated marked beneficial effects from phytohormone treatment. In contrast, earlier greenhouse experiments with a mixture of sand and an imported granulated peat moss failed to indicate significant improvement from this medium. Indeed, some injurious effects

were noted (7, 8). Increased survival in sand on treatment with 1000 p.p.m. indolylacetic acid was in agreement with the results of an earlier experiment (7).

It may be observed that the low rooting in sand only was rather surprising in the light of previous results under similar greenhouse conditions (7). There was, however, one difference in the propagation conditions in this and the former experiments. In the former the sand was watered heavily. In the present, watering was reduced and the sand maintained moist rather than thoroughly drenched. The results suggest that the moisture-sand relations recommended by propagators for many plants are not sufficiently moist for the successful propagation of Norway spruce cuttings in sand.

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MENDELIAN INHERITANCE OF CERTAIN PATHOGENIC CHARACTERS OF *PUCCINIA GRAMINIS TRITICI*[1]

By Thorvaldur Johnson[2] and Margaret Newton[3]

Abstract

Crossing and selfing studies with physiologic races of *Puccinia graminis Tritici* have shown that certain pathogenic characters are dominant to others. The "0" type of infection (absence of rust pustules) on the variety Kanred was found to be dominant to the "4" type (large rust pustules), so that when a race producing the "0" type was crossed with a race producing the "4" type the hybrid rust produced the "0" type. When the hybrid race was selfed, the "0" type occurred about three times as frequently in F_2 as the "4" type, a fact indicating that rust behaviour on this variety is governed by a single-factor pair. The "4" type of infection on the variety Mindum normally was dominant to the "1" type (very small pustules) and occurred about three times as frequently in F_2. The "1" type of infection on the emmer variety Vernal was dominant to the "4" type and recurred in some crosses, about 15 times as frequently in F_2 as the "4" type. Rust behaviour on this variety appears to be governed by duplicate factors, each factor being capable of exerting the same effect. Evidence derived from a study of the F_2 populations of two crosses between races 9 and 36 indicated that the factors governing rust behaviour on Kanred, Mindum, and Vernal, were different and were inherited independently of each other. In crosses in which the two parent races produced different infection types on the variety Marquis, the cytoplasm of the maternal parent race appeared to influence pathogenicity on this variety.

As a result of these studies it is concluded that despite the binucleate condition of stem rust in its uredial phase, the genes function as if they were present in a single diploid nucleus, and that, owing to fusion of the nuclei in the teliospore and subsequent meiotic divisions, independent segregation of factors occurs as in higher plants. The crossing of physiologic races and the selfing of the hybrids lead to various recombinations of existing pathogenic characters that may result in the formation of new physiologic races without involving the creation of pathogenic characters not possessed by the parent races.

Introduction

The identification of physiologic races of the cereal rusts is based on the fact that these races produce definite infection characteristics (infection types) on selected cereal host varieties. In *Puccinia graminis Tritici* Erikss. and Henn., identification of physiologic races is conducted by determining the infection types produced on 12 wheat varieties. Six rather well defined infection types designated as 0, 1, 2, 3, 4, and x occur on these varieties. These infection types, originally described by Stakman and Levine (9) are illustrated in Fig. 1. Because these infection types are relatively constant under ordinary greenhouse conditions, they may be regarded as characters of the rust organism and may, like other characters of living organisms, be subjected to inheritance studies when crosses are made between two physiologic races with sharply contrasted infection types on a given host plant. A study of the infection types produced on the variety Kanred (or Reliance) may serve as an example. On this variety many physiologic races produce either

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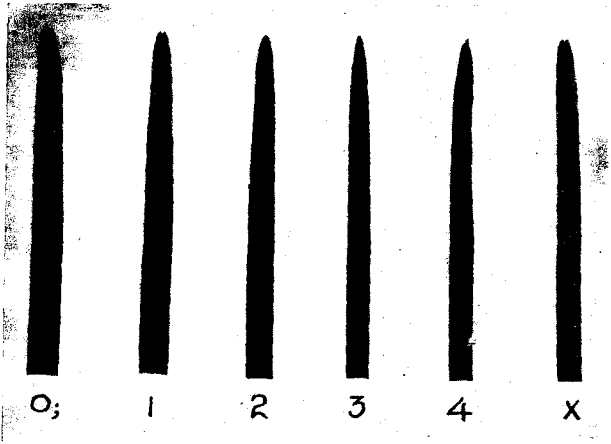

FIG. 1. *Six infection types caused by physiologic races of Puccinia graminis Tritici on differential wheat varieties.*

no visible signs of infection or, at most, minute necrotic flecks. This is known as a "0" type of infection. Other races produce large pustules: a "4" type of infection. If a race which is homozygous for the "0" infection type is crossed with a race homozygous for the "4" infection type, it is easy to demonstrate the dominance or recessiveness of this pair of contrasted characters by inoculating leaves of the variety Kanred with spores of the F_1 hybrid of the cross. If the F_1 hybrid race produces the "0" type of infection, it is clear that this type is dominant to the "4" type. Should a study of the inheritance of these characters in the F_2 generation be desired, the hybrid rust may be selfed in order to determine the frequency distribution of the two infection types in that generation. The same method of study may be applied to the infection types produced on any other host variety. In the study here reported, attention has been given chiefly to the inheritance of infection characteristics on the varieties Marquis, Kanred, Mindum, and Vernal. The methods used in crossing and selfing studies have been described in previous publications (5, 7). It is the purpose of the present paper to record and interpret the results of the crosses that have thus far been made between physiologic races of *Puccinia graminis Tritici*. Preliminary accounts of this work have appeared in earlier papers (4, 6).

Dominance of Certain Characters in F_1

The crosses and their F_1 progeny are recorded in Table I which also includes comments on the inheritance of those pathogenic characteristics by which the two parent races differed. Particular attention was given to the inheritance of the "0" and "4" infection types on Kanred, the "1" and "4" types on Mindum, and the "1" and "4" types on Vernal. The choice of these infection types was largely decided by the fact that several races were available that were homozygous for these characters.

In the crosses between races 9 and 36, and 9 and 52, reported in earlier papers (4, 5, 8), it was noted that, for Kanred, the "0" infection type produced by race 9 was dominant to the "4" type produced by races 36 and 52; that, for Mindum, the "4" type produced by race 9 was dominant to the "1" type produced by the other races; whereas, for Vernal, the "1" type produced by race 36 was dominant to the "4" type produced by race 9. These results are illustrated below by reference to a cross between races 9 and 36.

		Kanred	Mindum	Vernal
Parents	Race 9	0	4	4
	Race 36	4	1	1
F_1	Race 17	0	4	1

These findings raised the question whether these results are peculiar to crosses between the above-mentioned races or whether they are of general occurrence in crosses between races of wheat stem rust. The crosses recorded in Table I were made largely with the purpose of throwing light on this question.

Not all the races used in these crosses were homozygous for their infection types on all three varieties. A race homozygous for its infection type on Kanred might be heterozygous for its infection type on Mindum or *vice versa*. The conclusions derived from any cross have reference, chiefly, to the characters for which the parent races were homozygous.

Infection Types on Kanred.—In all except one of the 49 crosses between races homozygous for the "0" and "4" types of infection on Kanred, the "0" type proved dominant, and in all crosses in which both parent races produced a "4" type of infection on this variety the hybrid race also produced the same infection type. No crosses were made between two races both of which produced the "0" type. As far as can be judged from the crosses that have been made, it appears that the dominance of the Kanred "0" type is a common phenomenon in stem rust.

Infection Types on Mindum.—In crosses between races producing "1" and "4" types of infection on Mindum, the "4" type has been commonly, though not invariably, dominant. In the 31 crosses between races homozygous for

TABLE I

CROSSES BETWEEN PHYSIOLOGIC RACES OF *P. graminis Tritici*

Races crossed	Varieties on which parent races are homozygous for contrasted infection types	No. of crosses	Races in F_1	Comments on pathogenicity in F_1
9* × 36 36 × 9	Krd, Mnd, Ver	6 3	17 17	Krd "0" dominant to Krd "4" Mnd "4" dominant to Mnd "1" Ver "1" dominant to Ver "4"
9 × 15 15 × 9	Krd	1 1	9 9	Krd "0" dominant to Krd "4"
9 × 52 52 × 9	Krd, Mnd	1 2	9 9	Krd "0" dominant to Krd "4" Mnd "4" dominant to Mnd "1"
52 × 57	Krd	8	57	Krd "0" dominant to Krd "4"
52 × 120	Krd	2	57	Krd "0" dominant to Krd "4"
52 × 15	Mnd	4	15	Mnd "4" dominant to Mnd "1"
1 × 15 1 × 15	Krd, Mnd	2 1	9 17	Krd "0" dominant to Krd "4" Mnd "4" dominant to Mnd "1"
57 × 36	Krd, Ver	5	1	Krd "0" dominant to Krd "4" Ver "1" dominant to Ver "4"
36 × 36		1	36	
36 × 21	Krd	4	17	Krd "0" dominant to Krd "4" Mnd "4" dominant to Mnd "1"
36 × 15 36 × 15	Mnd, Ver	4 1	11 110	Mnd "4" dominant to Mnd "1" Ver "4" dominant in four crosses but infection type intermediate in one
36 × 120 36 × 120 36 × 120 36 × 120 36 × 120 120 × 36 120 × 36	Krd, Ver	1 5 3 1 1 1 1	155 136 157 158 172 136 158	Krd "0" dominant in 12 of 13 crosses Ver "1" dominant in 8 of 13 crosses; infection type intermediate in five crosses Mnd "1" appeared in 11 crosses, Mnd "4" in two
125 × 11 125 × 11	Mnd	1 1	11 → 126 →	Mnd "4" dominant to Mnd "1" Infection type on Mnd intermediate
36 × 51 51 × 36	Ma? Ver?	1 1	175 152	Ma "2" dominant to Ma "4" Ver "1" dominant to Ver "4" in one cross but infection type intermediate in the other

TABLE I—*Concluded*

CROSSES BETWEEN PHYSIOLOGIC RACES OF *P. graminis Tritici*—*Concluded*

Races crossed	Varieties on which parent races are homozygous for contrasted infection types	No. of crosses	Races in F_1	Comments on pathogenicity in F_1
36 × 14	Ma, Mnd	4	88	
14 × 36		1	14	
36 × 152	Ma	3	171	
152 × 36		3	152	
39 × 125	Ma	2	173	In all these crosses, one parent race produces a "1" or "2" type of infection on Marquis, the other a "4" type. In the hybrid progeny, infections on Marquis resemble those of the maternal parent in each cross.
39 × 125		1	174	
39 × 125		3	177	
39 × 125		1	39	
125 × 39		3	18	
125 × 39		2	56	
125 × 39		1	125	
125 × 39		1	34	

* *In all the crosses, the parent race placed first is the race on whose haploid pustules aecia were produced as a result of a transfer to them of the pycniospore-containing nectar of the other race.*

these characteristics, 30 produced the "4" infection type and one the indeterminate "x" type. In the 22 crosses in which both parent races produced the "1" type of infection, the F_1 hybrids also produced that infection type. Only in crosses of which one parent race was heterozygous for its infection type on Mindum did both infection types appear in the F_1 generation.

Infection Types on Vernal.—In the majority of crosses between races homozygous for the "1" and "4" infection types on Vernal, the "1" type proved dominant. In 34 such crosses, 27 gave rise to the "1" type while 7 produced an "x" (indeterminate) infection type. The "1" and "4" types of infection, both, appeared only in the F_1 progeny of crosses in which one parent race was heterozygous for the "1" type of infection, as in the cross 1 × 15 where race 1 was heterozygous. All the 19 crosses between races producing the "4" type on Vernal gave rise to F_1 progeny with the "4" infection type. Eleven crosses between races homozygous for the "1" type of infection produced only progeny with that infection type (36 × 36, 36 × 21, 36 × 152). The rather frequent appearance of the "x" type of infection in crosses between races homozygous for the "1" and "4" types seems to show that dominance of the "1" type is sometimes incomplete.

Infection Types on Marquis.—Crosses of races producing a "1" or "2" type of infection on Marquis with races producing a "4" type on this variety bring

TABLE II

INFECTION TYPES PRODUCED ON DIFFERENTIAL WHEAT VARIETIES BY THE PHYSIOLOGIC RACES OF *P. graminis Tritici* USED IN OR DERIVED FROM CROSSING AND SELFING STUDIES

Race	L.C.	Ma.	Krd.	Ko.	Arn.	Mnd.	Spm.	Kub.	Ac.	Enk.	Ver.	Kpl.
1	4	4−	0	3+	1=	1	1=	3+	3++	3	0;	1=
9	4	4−	0	3++	4−	4=	4=	4=	3++	3+	4±	1−
11	4−	4=	3++	3+	4=	4=	4=	3++	3++	3	1=	1=
14	4+	2−	1−	1++	3++	3++	3++	3++	3++	3	1=	0;
15	4	4−	4=	3++	4=	4=	4=	3++	3++	3++	4±	1=
17	4	4−	0	3+	4=	4=	4=	3++	3++	3	1=	1=
18	4	4−	4=	3++	1	1=	1−	3++	3++	3+	1−	1±
21	4	4	0	3++	4−	4−	4−	4=	3++	1=	0;	1=
29	4	4−	0	3	x++	x±	x+	x	x+	3	1−	1−
32	4	4=	4=	3+	x+	x±	x±	x−	x+	3	1=	1−
34	4+	4−	4−	4=	4	4=	4=	4±	3++	1=	0;	1±
36	4	4	4−	3++	1=	1=	0;	x	3++	3+	0;	1−
39	4−	2=	4=	3+	4+	3++	4−	4=	3++	4=	1=	1−
51	4	2=	3+	0;	0;	0;	0;	4	3++	3+	4−	0;
52	4	4	4−	4	1=	1=	1=	x±	4	4−	4+	1−
56	4	3+	3+	3+	1=	1=	1=	3+	3+	1=	1=	1−
57	4	4−	0	3+	1	1	1	4	3+	3	3	1
85	4	4−	0	3±	4−	4	4	4	4	3+	x	1
88	4	x	0	1±	4	4	4	4	4	3+	1	1
110	4	4−	3	3−	3+	3+	3+	3+	3+	3	x−	1
120	4	x	0	3=	4	4	4	4	4−	3+	3	1−
125	4±	4	4	4	0;	0;	0;	x	4	1=	0;	1−
126	4=	4=	3+	3+	x±	x++	x+	x−	x	1±	1=	1−
136	4	x	0	3=	0;	0;	0;	4−	4	4−	1−	1
152	4	1+	3−	0;	1	0;	0;	x−	3+	3+	0;	1−
155	4	x	0	3=	1	0;	0;	x	4	3+	0;	1
157	4	x	0	3=	1−	0;	0;	4	4	3+	x	1−
158	4	x	0	3=	4	4−	4−	4−	4−	3+	x	1
171	4	x±	3−	0;	1	0;	0;	x−	3+	3+	0;	1−
172	4	x	3−	2−	1−	0;	0;	4−	4	3	0;	1−
173	4	2	4−	3−	0;	0;	0;	4	4−	1−	0;	1
174	4	2	4−	3=	4−	x	x	4−	4−	1−	0;	1
175	4	2+	4	1±	1−	0;	0;	x	4−	3±	x	1−
177	4	2	4−	3−	1−	1−	1−	4−	3+	3	0;	1−

to light a kind of inheritance not encountered in the study of the infection characteristics on the other varieties. In 25 of 27 such crosses, the F_1 progeny produced on Marquis an infection type closely resembling that of the maternal parent race, that is, the race on whose haploid pustules aecia were formed. The F_1 progeny arising from one side of such a cross produced a "1" or "2" type of infection whereas that from the opposite side produced a "4" type or occasionally an "x" type so heavy that it bordered on a "4" type of infection. This behaviour, in crosses, of the infection types on Marquis has been described for the cross race 36 × race 14 in previous papers (4, 5, 6, 8) in which it was attributed to the influence of the cytoplasm of the maternal parent race. Its occurrence in crosses between other races suggests that it may be a rather general phenomenon. A similar type of inheritance has been shown to govern the infection types on Joanette strain in crosses between physiologic races of *Puccinia graminis Avenae* Erikss. and Henn. (3).

Selfing Studies

General Survey of Selfing Studies

Evidence bearing on the dominance or recessiveness of the above-mentioned infection characteristics may be obtained not only from crossing studies but also from the selfing of physiologic races. If, in the selfing of physiologic races from many different sources, it should be found that a certain characteristic, such as the "4" infection type on Kanred, always reproduces itself in the progeny whereas another characteristic, such as the "0" infection type, frequently produces types differing from itself, it is natural to suppose that the former occurs only in a homozygous state while the latter may exist in either a homozygous or heterozygous condition. In this example, the "0" infection type would be assumed to be dominant to the "4" type.

Since hybridization studies with stem rust were first undertaken by the writers, they have selfed 66 cultures comprising 24 physiologic races. Data on the homozygosity or heterozygosity of these cultures for their pathogenic characteristics on the varieties Kanred, Mindum, and Vernal are presented in Table III.

TABLE III

HOMOZYGOSITY OR HETEROZYGOSITY OF 66 SELFED CULTURES OF WHEAT STEM RUST FOR CERTAIN PATHOGENIC CHARACTERS

Pathogenic character	No. cultures selfed	Number homozygous	Number heterozygous
Kanred "4"	26	25	1
Kanred "0"	40	17	23
Mindum "1"	28	24	4*
Mindum "4"	36	13	23
Mindum "x"	2	0	2
Vernal "4"	22	17	5**
Vernal "1"	44	21	23

* *Two of these cultures may have been homozygous.*
** *Three of these cultures may have been homozygous.*

The data supplied in Table III provide support for the hypothesis that the "4" type of infection on Kanred, the "1" type on Mindum and the "4" type on Vernal, are recessive characteristics. Theoretically, all the cultures showing these characteristics should have proved homozygous. Actually, a few showed indications of heterozygosity. The exceptions shown in Table III are probably the result of undetected impurities in the selfed races, accidental introduction of foreign pycniospore-bearing nectar or mutation.

The Distribution of Pathogenic Characters in F_2

In several of the crosses mentioned in Table I, one or more F_1 hybrid cultures have been selfed with the purpose of discovering the distribution in the F_2 generation of characters (infection types) that were dominant or reces-

sive in the cross. The most extensive selfing studies made, thus far, have been carried out with two separate crosses between races 9 and 36. These two races differ in their pathogenic characteristics on the variety Kanred, on the *durum* varieties Arnautka, Mindum, Spelmar and Kubanka, and on the emmer variety Vernal (Table II). In these studies, each F_2 culture originated from a single aecial cup selected at random from numerous aecial pustules on several barberry plants infected with the F_1 hybrid race—race 17. The frequency distribution in F_2 of the contrasted infection types on the varieties Kanred, Mindum, and Vernal is shown in Table IV. The significance of the ratios was determined by the Chi-square (χ^2) method according to the formula $\chi^2 = \Sigma \left[\frac{(a - t)^2}{t} \right]$, where a represents the actual and t the theoretical frequencies of the contrasted infection types.

TABLE IV

FREQUENCY DISTRIBUTION IN F_2 OF THE CROSS, RACE 9 × RACE 36, OF THE CONTRASTED INFECTION TYPES ON KANRED, MINDUM, AND VERNAL

Infection type	Cross I	Cross II	Crosses I and II combined
Kanred "0"	96	131	227
Kanred "4"	30	68	98
Ratio	3.2 : 1	1.9 : 1	2.3 : 1
χ^2	0.0952	8.8715	4.6334
P	0.75	0.01	0.03
Mindum "4"	89	142	231
Mindum "x"	0	11	11
Mindum "1"	37	46	83
Ratio	2.4 : 1	3.3 : 1	2.9 : 1
χ^2	1.2804	0.3866	0.0531
P	0.25	0.52	0.80
Vernal "1"	120	187	307
Vernal "x"	4	3	7
Vernal "4"	2	9	11
Ratio	20 : 1	15.6 : 1	17 : 1
χ^2	0.4874	0.0138	0.2778
P	0.49	0.90	0.58

It is clear from a study of Table IV that the infection types that proved dominant in the cross reappeared much more frequently in the F_2 generation than the recessive infection types. In calculating the ratios, the "x" type infections, which occurred occasionally on Mindum and Vernal, were grouped together with the "4" type. The ratios thus obtained approximate rather closely to a 3 : 1 for the infections on Kanred and Mindum and to a 15 : 1 ratio for those on Vernal. To test the goodness of fit to these ratios, the values of χ^2 and P were calculated for each cross as well as for the two crosses combined.

Taking the 5% point as a criterion of significance, the P values, with one exception, provide support for the hypothesis that the behaviour of the rust

on Kanred and on Mindum is in each case governed by a single pair of Mendelian factors and that the infection characteristics on Vernal are governed by two pairs of factors (duplicate factors). The exception is the distribution of infection types on Kanred in cross II.

TABLE V

A TEST (ON THE F_2 POPULATIONS OF TWO CROSSES BETWEEN RACES 9 AND 36) OF THE GOODNESS OF FIT OF THE DISTRIBUTION OF THE INFECTION TYPES ON KANRED AND VERNAL AND ON MINDUM AND VERNAL TO THEORETICAL RATIOS CALCULATED ON THE ASSUMPTION THAT THE RUST BEHAVIOUR ON KANRED AND MINDUM IS GOVERNED BY SINGLE FACTOR PAIRS AND THAT ON VERNAL BY TWO-FACTOR PAIRS

Infection types		Genotypes		Theor. ratio	Cross I		Cross II		Cross I and II combined	
					Actual distribution	Theor. distribution	Actual distribution	Theor. distribution	Actual distribution	Theor. distribution
Kanred	Vernal	Kanred	Vernal							
"0"	"1"	A	C and/or D	45	91	88.6	121	139.9	212	228.5
"4"	"1"	a	C and/or D	15	29	29.5	66	46.6	95	76.2
"0"	"x" "4"	A	c and d	3	5	5.9	10	9.3	15	15.2
"4"	"x" "4"	a	c and d	1	1	2.0	2	3.1	3	5.1
					χ^2 = 0.711 P = 0.87		χ^2 = 10.727 P = 0.01-0.02		χ^2 = 6.696 P = 0.09	
Mindum	Vernal	Mindum	Vernal							
"x" "4"	"1"	B	C and/or D	45	84	88.6	145	130.9	229	228.5
"1"	"1"	b	C and/or D	15	36	29.5	42	46.6	78	76.2
"x" "4"	"x" "4"	B	c and d	3	5	5.9	8	9.3	13	15.2
"1"	"x" "4"	b	c and d	1	1	2.0	4	3.1	5	5.1
					χ^2 = 2.309 P = 0.5		χ^2 = 1.099 P = 0.8		χ^2 = 0.364 P = 0.95	

Further confirmation of this hypothesis and an indication that these factors are inherited independently are obtained from the data presented in Table V. For this table, a calculation was made of the expected frequencies of all possible combinations of infection types on Kanred and Vernal and on Mindum and Vernal, on the assumption that in each case the infection types on the two hosts were inherited independently of each other. As the infection types on Kanred and Mindum appear to be governed by a single-factor pair and those on Vernal by a two-factor pair, the data should conform to a tri-hybrid ratio in which, for example, in a population of 64 F_2 cultures, the "0" type on Kanred and the "1" type on Vernal would be associated 45 times, the "4" type on Kanred and the "1" type on Vernal 15 times, the "0" type on Kanred and the "1" type on Vernal three times, and the "4" type on Kanred and the "4" type on Vernal once. The agreement of the theoretical and actual ratios is very close for the Mindum-Vernal infection types in both crosses (P values 0.5 and 0.8) and for the Kanred-Vernal infection types in cross I

(P values 0.87) but is poor for Kanred-Vernal in cross II (P value 0.01 to 0.02). In spite of the absence of statistical evidence in this last instance, it seems probable that the pathogenic behaviour of the rust is governed by one-factor pairs for Kanred and Mindum and by a two-factor pair for Vernal and that these factors are assorted independently of each other.

TABLE VI

ACTUAL AND THEORETICAL DISTRIBUTION OF PHYSIOLOGIC RACES IN THE COMBINED F_2 GENERATIONS OF TWO CROSSES BETWEEN RACES 9 AND 36, THE THEORETICAL DISTRIBUTION BEING BASED ON THE ASSUMPTION THAT SINGLE-FACTOR PAIRS GOVERN RUST BEHAVIOUR ON THE VARIETIES KANRED AND MINDUM AND THAT TWO PAIRS OF FACTORS GOVERN RUST BEHAVIOUR ON THE VARIETY VERNAL

Parent Races				F_1	F_2				
					Genotypes	Race	Theor. distribution	Actual distribution	
					A B C D				
		Race 9			A B c D	17	171.4	165[1]	
					A B C d				
Hosts	*Krd*	*Mnd*	*Ver*		a B C D				
					a B c D	11	57.1	64[2]	
Genotype	AA	BB	ccdd	*Krd Mnd Ver*	a B C d				
					A b C D				$\chi^2 = 11.374$
Inf. type	"0"	"4"	"4"	Aa Bb CcDd	A b c D	1	57.1	47	
				Race 17	A b C d				$P = 0.13$
		Race 36		"0" "4" "1"	a b C D				
					a b c D	36	19.0	31	
Hosts	*Krd*	*Mnd*	*Ver*		a b C d				
					A B c d	9	11.4	11[3]	
Genotype	aa	bb	CCDD		A b c d	57	3.8	4	
					a B c d	15	3.8	2[4]	
Inf. type	"4"	"1"	"1"		a b c d	52	1.3	1	

[1] *In this group are included six cultures of race 29 which differs from race 17 by producing an "x" infection type on Mindum instead of a "4" type.*

[2] *In this group are included five cultures of race 32 which differs from race 11 by producing an "x" infection type on Mindum instead of a "4" type.*

[3] *In this group are included six cultures of race 85 which differs from race 9 by producing an "x" infection type on Vernal instead of a "4" type.*

[4] *In this group is included one culture of race 110 which differs from race 15 by producing an "x" infection type on Vernal instead of a "4" type.*

To test this theory further a comparison was made of the theoretical distribution of physiologic races in the F_2 generation of crosses I and II with the actual distribution of the races. The results are shown in Table VI. This table gives (i) the supposed genetic constitution of the parent races and of the F_1 and F_2 hybrids, (ii) the theoretical distribution of the races expected on the assumption that dominance is complete, and (iii) the actual distribution. According to the theory outlined above, the various possible tetra-hybrid combinations should give rise to the following races: 1, 9, 11, 15, 17, 36, 52, and 57. *Actually, all these races did occur in the F_2 population but so did four others,* namely, races 29, 32, 85, and 110. All these, however, bear a rather close

resemblance to certain of the expected races, differing only by their tendency to produce an "x" type of infection on certain differential varieties. Thus race 29 is identical with race 17, and race 32 with race 11, except for a tendency to produce an "x" infection type on certain *durum* varieties including Mindum. Similarly races 85 and 110 resemble races 9 and 15 respectively except for a disposition to produce an "x" infection type on Vernal. The "x" infections on the *durum* varieties are perhaps the result of environmental conditions at the time of race identification rather than genotypic differences between cultures, as this type of infection was confined to one group of cultures identified within a period of less than one month. Owing to the resemblance of the races characterized by "x" infections to others identified much more frequently they have, in Table VI, been grouped together with the races to which they bear the closest resemblance.

Granting this modification, the actual distribution of the physiologic races in the F_2 population conforms in most instances rather closely to theoretical expectation, as will be seen in Table VI. As a result, however, of race 36 occurring more frequently and race 1 less frequently than expected, the value of χ^2 is rather high and the value of P rather low. The P value of 0.13 is not, however, low enough to exclude the possibility of the correctness of the proposed scheme of inheritance.

Discussion

Interpretation of the experimental results presented in this paper leads to the conclusion that the pathogenic properties of physiologic races of *Puccinia graminis Tritici* are inherited, for the most part, in accordance with Mendelian laws of inheritance. There is evidence for the phenomena of dominance, recessiveness, and independent segregation of the factors governing rust behaviour. There is evidence that some pathogenic characters are governed by a single-factor pair, others by more than one factor pair. Genotypically, a physiologic race must be considered to contain many genes or factors that in one way or another affect its pathogenicity. Some of these factors appear to govern the behaviour of the rust on certain differential varieties without apparently affecting rust behaviour on other varieties. There is evidence that the single-factor pair governing the behaviour of the rust on the variety Kanred is different from the single-factor pair governing rust behaviour on the variety Mindum, and it is clear, furthermore, that the two pairs of factors governing the infection types on Vernal differ from both. This does not, of course, imply that a given single-factor pair affects only the rust behaviour on a single variety. On the contrary, it is likely that a given factor pair governs rust behaviour on many closely related varieties. In several of the crosses studied it seemed manifest that rust behaviour on the varieties Arnautka, Mindum, and Spelmar was governed by the same factor pair.

It is not to be assumed, as a result of the presence in certain crosses of 3 : 1 ratios for rust behaviour on Kanred and Mindum and a 15 : 1 ratio for rust behaviour on Vernal, that such ratios are of universal occurrence in the selfing

of heterozygous races. When duplicate factors govern rust behaviour, as appears to be the case in Vernal, different lines descended from the same cross may produce different ratios. In the scheme of inheritance outlined in Table VI, F_2 lines with genotypes Ccdd and ccDd should, when selfed, produce 3 : 1 ratios while F_2 lines with the genotype CcDd should produce 15 : 1 ratios. The few selfing studies carried out with F_2 lines do actually suggest the existence of both ratios.

The question of dominance is of interest in view of the dikaryotic condition of the uredial phase of stem rust. That the phenomenon of dominance is expressed in the uredial stage is shown by crossing and selfing studies alike, and this applies equally to urediospore-colour and pathogenicity. As shown elsewhere (2, 4) red, grayish-brown, or orange spore colour is dominant to white. Among pathogenic characters that have been studied, the "0" infection type on Kanred is dominant to the "4" type; the "4" infection type on Mindum is dominant to the "1" type; the "1" type of infection on Vernal is dominant to the "4" type. In oat stem rust, it has been shown (3) that red urediospore colour is dominant to orange, and that certain infection types on the varieties White Tartar and Richland are dominant to others.

These results make it evident that the binucleate condition of the rust organism does not in any way interfere with the expression of dominance. That this fact is not generally realized is shown by the assertion of Dodge (1) that dominance cannot be expressed in a dikaryophyte. In view of the results enumerated above it would seem that the distinction sometimes made between an uninucleate, diploid organism and a dikaryotic one is purely academic. This distinction has no practical basis if allelomorphic genes function in the same manner whether they are both present in a single diploid nucleus or one in each of the two haploid nuclei of a dikaryophyte. Furthermore, there can be no practical reason for not applying the term "hybrid" to a new dikaryotic combination when it is realized that the genes present act is if they were contained in a true diploid nucleus. When it is further realized that the dikaryon fuses to form a true diploid nucleus (in the teliospore) and that this diploid nucleus divides meiotically, it becomes apparent that the analogy with uninucleate organisms is complete except for the fact that the two nuclei of the dikaryophyte do not fuse during a certain phase of the life cycle of the rust.

One curious characteristic of many crosses between the physiologic races of stem rust of wheat and of oats, is the tendency of the F_1 hybrids from opposite sides of the same cross to differ in certain pathogenic characters. As the nuclear material in such reciprocal hybrids should be identical, it does not seem possible to explain this phenomenon in terms of gene action. It is possible that the pycniospores brought over from another race lose their scanty amount of cytoplasm by the time they reach the aecial primordium where diploidization takes place, so that the resultant dikaryon contains nuclear material from both parent races but cytoplasm from only one—the maternal race. The cytoplasmic influence (if such it is) is expressed only in

certain pathogenic characteristics but leaves others unaffected. In crosses between physiologic races of wheat stem rust it is manifest if the parent races differ in their infection types on the variety Marquis, and can be detected only through the infection types on this variety. In crosses between races of oat stem rust it may be discerned in the infection types on Joanette strain (3). In these instances, the influence of the cytoplasm apparently overcomes or obscures the effects of nuclear factors.

In view of the fact that rust behaviour on different wheat varieties may be governed by different factors inherited independently, it can be readily understood why many physiologic races may occur in the progeny of a cross between two races. If the two parent races differ pathogenically on four differential varieties, and if the factors governing these differences are inherited independently, it is clear that, under circumstances of complete dominance, 16 phenotypic classes, that is, physiologic races, may be expected to occur in the F_2 generation. In this generation, the pathogenic characters of the parent races are recombined in various ways without, however, necessarily involving the creation of new pathogenic characters. It would seem, therefore, that crossing of races does not necessarily change the pathogenic ability of the rust although new combinations of pathogenic characters present in the parents are brought into being.

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NOTE ON THE PROPAGATION BY CUTTINGS OF WHITE PINE AND WHITE SPRUCE[1]

By J. L. Farrar[2] and N. H. Grace[3]

Throughout the past three years (1938–1940) the authors have been interested in the vegetative propagation of conifers important in forestry. Preliminary work on a number of spruces and pines indicated that Norway spruce (*Picea Abies* (L.) Karst.) was less difficult to root from cuttings than the others. Consequently much intensive work has been done with this species in the expectation that the discovery of a successful method of rooting Norway spruce would lead to a solution of the problem of rooting our important native conifers (2). Experiments based on this work have now led to the rooting of white spruce (*Picea glauca* (Moench) Voss.) and white pine (*Pinus strobus* L.), and this note gives a preliminary account of the methods and the results obtained. A full report will appear in this Journal at a later date.

The cuttings were collected from a large group of 10- to 15-year-old trees, and were from 5 to 10 cm. long, and of the current year's growth. They were planted in outside frames protected by lath and factory cotton shades, the propagation media rested directly on the ground.

The propagation medium proved to be the most important single factor affecting rooting, a mixture of peat and sand being vastly superior to sand alone. Furthermore, a well-decomposed native peat of sedge origin (1, 2) was found to result in much better rooting than the European peat moss of sphagnum origin commonly used in horticulture. It also had a very pronounced effect in stimulating buds to produce new shoots. Of only slightly less importance was the time of collecting the cuttings. Late July was the most favourable period for white spruce, whereas mid-August was most favourable for white pine. Indolylacetic acid mixed with talc and applied to the base of the cuttings did not have any marked effect on rooting.

Under the optimum combination of the conditions mentioned, white spruce was rooted 80 to 90% on the average, with individual groups of 10 cuttings attaining 100%. Likewise white pine was rooted 50 to 60% on the average, with individual groups of ten cuttings attaining 90%.

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EFFECT OF CROWN GALL FORMATION ON THE CHEMICAL COMPOSITION OF BEETS[1]

By A. C. Neish[2] and Harold Hibbert[3]

Abstract

Tumours were produced on *Beta vulgaris* (beet) by inoculation with *Phytomonas tumefaciens* (crown gall organism). Analyses were made of tumorous tissue, normal tissue of tumorous beets, and the tissue of healthy beets (not inoculated) dried at 60° C. in a vacuum oven. The constituents determined quantitatively were glucose, fructose, sucrose, starch, cellulose, lignin, pentosans, uronic anhydride (in holocellulose), total uronic anhydride, pectins, ether extract, crude protein, total ash, tannins, free phenolics, methoxyl, and solid matter. The results are discussed with respect to *(a)* the effect of the bacterial stimulus on the physiology of the plant and *(b)* the resulting changes in chemical composition.

The bacteria exert a local stimulus causing the cells to proliferate and form a tumour. Sucrose is converted into cell wall and protoplasm in the tumour cells. This sucrose is obtained from the normal tissue of the root by the tumour cells. This normal tissue is not stimulated by the bacterium to convert sucrose into other materials. Owing to this increased synthetic activity of tumour cells, an infected root contains more solid matter than a non-infected one, but less sucrose. The composition of the cell wall is essentially the same in tumorous and normal tissue. The conversion of sucrose to protein is a major metabolic process in tumours on beet roots. Ethanolysis of beet root indicates that the lignin present is probably of the same general nature as the lignin in woods.

Introduction

Strohmer and Stift (17) found that the galls of sugar beets had a higher content of ash, protein, and moisture but a lower content of sugar than normal roots.

Levine (11) found that the total weight of infected beets, grown in fertile soil, was greater than the total weight of non-infected beets and also that the galls developed to the greatest extent on the beets that were the best nourished. Brown and Quirk (4) found the total acid content of tumour juice to be greater than that of juice from normal tissue although the pH of the former was more on the alkaline side. They measured the redox potential with a gold electrode and found the tumour juice to be negative with respect to the normal juice. Binet and Magrou (3) determined reduced glutathione in *Pelargonium zonale* inoculated with *P. tumefaciens*. When the galls were four months old they contained 316 to 437 mg. of glutathione per 100 gm. of fresh tissue; the leaves contained 101 to 122 mg., green stems 83 to 142 mg., and the terminal growing point 500 to 1000 mg. per 100 gm. of fresh tissue. Following necrosis of the gall the glutathione disappeared; thus the latter appears to be associated

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with rapid tissue proliferation. Berthelot and Amoureux (2) showed that beet crown galls contained five times as much reduced glutathione, twice as much ascorbic acid, 20% more nitrogen and 20% less ash than normal tissue. They found that differences in total dry matter, sugars, potassium, and phosphorus were slight and irregular.

Townsend (18) analyzed fresh galled beets (after removal of galls) and healthy beets and found that the healthy beets contained 50% more sucrose than the galled beets. Nagy, Riker, and Peterson (12) found that the galls contained more glutathione than did adjacent tissues but not so much as did the growing tips. They found the galls of sugar beets contained 2.9% total nitrogen as compared with 0.9% in normal tissue. Also, the fresh galls contained 42% of non-reducing sugar (sucrose) as compared with 70% in the normal tissue, dry weight basis. The galled tissue of beets was found to have a somewhat higher content of starch, cellulose, pentosan, ash, and ether extract but little difference was found in uronic acid content. They made similar analyses of galls on tomato and raspberry stems and found the galls to be lower in cellulose and pentosans than the contiguous tissue. These plants are not so succulent as the beet root. Extracts from galls (on tomato) rapidly destroyed tyrosine but stem extracts did not. Catalase, oxidase, and peroxidase activity on a wet-weight basis were 160, 130, and 120% higher, respectively, in galls than in normal tissues. The same figures calculated on the basis of total nitrogen are 86, 73, and 57%, respectively.

Klein and Zeise (9) found the catalase activity in crown gall of beets to be greatly increased over that of contiguous tissue. They also found (10) in galls on horse radish that the increase in catalase was paralleled by an increase in peroxidase.

It is interesting to note that Rasnowski (16) observed that lignification of sugar beets was a hereditary property and that a low sugar content was associated with a high degree of lignification.

The present investigation provides further data on the chemical composition of crown galls on beets and the contiguous tissue, also on that of non-infected controls. It is found that by calculating the analytical data on various bases it is possible to draw conclusions concerning the physiology of the tumours.

Experimental

Preparation of Material

Beets of the variety "Extra Early Flat Egyptian" were grown in pots in a greenhouse, using fairly fertile soil. One hundred plants were raised under the same conditions. Sixty of these were inoculated with *Phytomonas tumefaciens* and the other forty kept as controls.

The seeds were soaked overnight in water and then planted in a flat. When the seedlings reached a height of about two and one-half inches and had developed true leaves they were transplanted into 6-in. pots, one to each pot. When the storage organ (root) reached about one-quarter of an inch in diameter the plants were inoculated on one side of the crown by stabbing

several times with a needle previously dipped into a two day old culture of *P. tumefaciens* grown on beef-peptone agar.

The plants were allowed to develop until the storage organs (roots) reached a diameter of 1½ to 2½ in. (the maximum size reached by this variety under these conditions). The tumours were well developed, being almost equal in size to the rest of the storage organ. The beets were then harvested, the tops and branch roots being removed leaving only the storage organ which was then scrubbed in running water with a test-tube brush until free from soil. The tumours were then removed from the infected beets. Since inoculations were made on one side only, the tumours were present on that side as a swelling which could be readily separated by cutting along a line that marked the natural contour of the beet root. The tumorous tissue did not differ markedly from the normal tissue in macroscopic appearance. The tumours were smooth and firm except at the point where the inoculations were made; here they were puckered. There was no sign of rot nor secondary infection in the beets taken for analysis.

The tissue was cut into thin slices about 2 mm. thick and dried in a vacuum oven at 60° C. for three days. The dried tissue was then ground in a porcelain mortar to give a powder fine enough to pass a 60-mesh screen. Fresh and dry weights were recorded. In this way three samples were obtained.

(*a*) Healthy beets (controls),

(*b*) Normal tissue from tumorous beets,

(*c*) Tumorous tissue.

These are composite samples: (*a*) represents 40 individual beets and (*b*) and (*c*) represent 50 individual beets.

Table I shows the fresh and dry weights of these samples.

TABLE I

FRESH AND DRY WEIGHTS OF SAMPLES

Type of sample	Fresh weight, gm.	Dry weight, gm.	Percentage of dry matter
Healthy beets	719.0	91.9	12.78
Normal tissue of tumorous beets	729.9	71.6	9.81
Tumorous tissue	574.2	59.6	10.38

Analysis of Samples

The dried samples were analyzed for the following substances by the methods indicated.

1. *Sucrose, fructose, and glucose.* Copper reduction method of van der Plank (15). It was found that inversion of the sucrose with invertase, takadiastase, and dilute hydrochloric acid yielded substantially the same result.

2. *Starch.* Takadiastase method as outlined by Denny (6).

3. *Crude protein.* Kjeldahl nitrogen was multiplied by the factor 6.25. The nitrogen was determined according to the method described by the Association of Official Agricultural Chemists (1, p. 279).

4. *Ether extract.* Method of the Association of Official Agricultural Chemists (1, p. 279).

5. *Total ash.* Method of the Association of Official Agricultural Chemists (1, p. 278).

6. *Tannins.* A special permanganate titration of an aqueous extract before and after adsorption of tannins on kaolin was employed (as described by the Association of Official Agricultural Chemists (1, pp. 155–156)).

7. *Pectic substances.* By precipitation as calcium pectate (Nanji and Norman (13)).

8. *Cellulose.* Schorger's method as described by Dorée (8).

9. *Uronic anhydride.* This was calculated from the carbon dioxide given off on boiling with hydrochloric acid (12%) following the method of Dickson, Otterson, and Link (7). It was determined both on total material and on holocellulose.

10. *Holocellulose* was prepared by the method of Van Beckum and Ritter (19). This material consists of cellulose and hemicellulose free from lignin. The comparatively high yield obtained with beet tissue indicates that it may contain the protein as well.

11. *Free phenols* were estimated in the ether extract using the colorimetric method of Deichmann and Scott (5).

12. *Pentosans.* The method of Kullgren and Tyden (Dorée (8, p. 364)), was used with the holocellulose.

13. *Acid lignin* was determined by Schwalbe's method as described by Dorée (8, p. 349).

14. *Ethanolysis of beet root* was found to give oils with a methoxyl content of 11 to 20%, so it was concluded that lignin in beets is not an artifact formed from carbohydrates.

15. *Methoxyl* was determined, by the method of Peniston and Hibbert (14), in *(a)* total tissue, *(b)* the residue after extraction with ether, and *(c)* the residue after extraction with ether and hot 0.5% aqueous ammonium oxalate. The value for *(c)* should represent the methoxyl of the lignin.

The results of these analyses are given in Table II, expressed as percentage of dry weight. From these values and those given in Table I it was possible to calculate the results on a fresh weight basis (Table III) and on the basis of amount of material in an average individual beet root (Table IV), and also to calculate the ratio of the substance in question to the crude protein (Table V). These results are discussed in detail in the next section.

Discussion

The results in Table II are expressed as percentage of the dry matter. Examination of these results shows that on this basis the tumours have a higher content of crude protein, ether extract, starch, pectins, pentosans, cellulose, lignin, methoxyl, polyuronides (of holocellulose), and total ash than healthy beets. The latter have a considerably higher content of sucrose than the tumours; the differences in reducing sugars are slight. Pectins and total uronic anhydride are present in lowest amount in the normal tissue of tumorous beets but otherwise this tissue is intermediate in composition between healthy beets and tumorous tissue. The presence of tannins and free phenols is doubtful. Most of the methoxyl is present in an insoluble form, presumably as lignin.

TABLE II

COMPOSITION OF NORMAL AND TUMOROUS TISSUE OF BEETS—RESULTS EXPRESSED AS PERCENTAGE OF TOTAL DRY WEIGHT

Constituent	Healthy beets	Normal tissue of tumorous beets	Tumorous tissue
Total ash	9.65	13.46	13.46
Free glucose	1.00	0.82	1.18
Free fructose	0.56	1.03	0.72
Sucrose	47.92	29.66	12.30
Starch	1.18	2.41	2.48
Ether extract	1.56	1.87	2.57
Crude protein	10.39	12.12	20.03
Pectins	9.69	8.00	13.15
Uronic anhydride (in holocellulose)	5.06	6.06	7.77
Pentosans (in holocellulose)	1.98	2.34	2.65
Cellulose	6.64	7.65	9.05
Acid lignin	4.06	4.88	5.97
Total of solid matter accounted for	*99.69*	*90.30*	*91.03*
Total uronic anhydride	12.47	11.36	12.93
Total methoxyl	0.813	0.920	1.264
Methoxyl of material insoluble in anhydrous ether	0.807	0.916	1.259
Methoxyl of material insoluble in ether and hot aqueous ammonium oxalate (0.5%)	0.704	0.850	1.064
Tannins	Less than 0.1	Less than 0.1	Less than 0.1
Free phenols*			

* *Presence doubtful.*

Summation of the results in Table II shows the solid matter accounted for to be:

(*a*) For healthy beets, 99.69%,

(*b*) For normal tissue of tumorous beets, 90.30%,

(*c*) For tumorous tissue, 91.03%.

In making this summation total uronic anhydride and methoxyl are not included since they come from pectins, uronic anhydride (in holocellulose), and lignin, which already have been included.

Thus practically all the dry matter of the healthy beets has been accounted for but only 90% of that of the infected beets.

The variation in the sucrose content is the most marked result. Naturally when one substance varies so widely, some other component or components of the tissue must necessarily show a variation in the opposite direction when expressed as percentage of dry matter. Examination of the results in Table II therefore do not indicate the conditions existing in the living tissue. To interpret these results in the light of chemical changes in composition associated with tumour formation they must be expressed in various ways.

TABLE III

COMPOSITION OF NORMAL AND TUMOROUS TISSUE OF BEETS EXPRESSED AS PERCENTAGE OF TOTAL FRESH WEIGHT

Constituent	Healthy beets	Normal tissue of tumorous beets	Tumorous tissue
Total ash	1.23	1.32	1.35
Free glucose	0.13	0.08	0.12
Free fructose	0.07	0.10	0.07
Sucrose	6.12	2.91	1.28
Starch	0.15	0.24	0.26
Ether extract	0.20	0.18	0.27
Crude protein	1.33	1.19	2.08
Pectins	1.24	0.78	1.36
Total uronic anhydride	1.59	1.11	1.34
Uronic anhydride (in holocellulose)	0.65	0.59	0.81
Pentosans (in holocellulose)	0.25	0.23	0.27
Cellulose	0.85	0.75	0.94
Acid lignin	0.52	0.48	0.62
Total methoxyl	0.10	0.09	0.13
Methoxyl insoluble in anhydrous ether	0.10	0.09	0.13
Methoxyl insoluble in anhydrous ether and ammonium oxalate (0.5%)	0.09	0.08	0.11
Tannins*			
Free phenols*			

* *Presence doubtful.*

In Table III the results are expressed as percentage of the fresh (undried) weight. Since this material is approximately 90% water it is apparent that comparing the amounts of a substance in equal weights of fresh tissue is essentially the same as comparing the amounts in equal volumes of fresh tissue. Therefore the results in Table III permit comparison of the concentrations of the substances *in the living tissues* of tumorous and healthy beets.

Table IV expresses the results as amount of the substances in an average individual beet root and enables one to obtain a measure of the effect of *Phytomonas tumefaciens* on the beet root as a whole.

Table V gives the ratio of substance to crude protein (on weight basis). Since the crude protein can be considered as a measure of the living protoplasm in this material, these figures show the amount of substance present relative to the protoplasm.

TABLE IV

AMOUNT OF MATERIAL IN AN AVERAGE INDIVIDUAL UNDRIED BEET ROOT (EXPRESSED IN GRAMS)

Constituent	Healthy beet root	Normal tissue of tumorous beet root	Tumorous tissue of beet root	Total in tumorous beet root
Average fresh weight	17.97	14.59	11.48	26.07
Average dry weight	2.297	1.432	1.192	2.624
Total ash	0.222	0.193	0.157	0.350
Free glucose	0.023	0.012	0.014	0.026
Free fructose	0.013	0.015	0.009	0.024
Sucrose	1.101	0.425	0.147	0.572
Starch	0.027	0.034	0.029	0.063
Ether extract	0.036	0.027	0.031	0.058
Crude protein	0.239	0.173	0.239	0.412
Pectins	0.222	0.115	0.157	0.272
Total uronic anhydrides	0.288	0.163	0.154	0.317
Uronic anhydride (in holocellulose)	0.116	0.088	0.093	0.181
Pentosans (in holocellulose)	0.045	0.033	0.032	0.065
Cellulose	0.152	0.109	0.108	0.217
Acid lignin	0.093	0.070	0.071	0.141
Total methoxyl	0.020	0.013	0.015	0.028
Methoxyl insoluble in anhydrous ether	0.019	0.013	0.015	0.028
Methoxyl insoluble in anhydrous ether and ammonium oxalate (0.5%)	0.016	0.012	0.013	0.025

The average infected beet is heavier than the non-infected beet and contains a greater total amount of solid matter (Table IV). This agrees with the work of Levine (11). However the average infected beet contains only about one-half as much sucrose as the average healthy beet, although it contains more of all the other substances. This indicates that the crown gall organism stimulates the beet root to produce these substances from sucrose. Both protoplasmic and cell wall materials are formed.

The concentration of the ash elements is greatest in the tumorous tissue (Table III). Possibly it is because these are required to neutralize the acids which Brown and Quirk (4) have shown to be present in higher concentrations in tumours. The concentration of sucrose is lowest in tumours, being only one-fifth the concentration in the healthy living tissue of non-infected beets, and less than one-half the concentration present in the normal tissue of tumorous beets (Table III). It is interesting to note that the concentrations of glucose and fructose show no marked variation and that starch is actually present in increased concentration in the tumours (Table III). A noteworthy fact (Table III) is that the *normal tissue of tumorous beets* has a lower concentration of protoplasmic (crude protein, ether extract) and cell wall substances (lignin, methoxyl, cellulose, uronic anhydride, pentosans, and pectin) than either tumorous tissue or healthy tissue of non-infected beets. It also contains the lowest concentration of solids (Table I). From these results it would seem that the tumorous tissue robs the normal tissue of the same root of its stored sucrose and converts this into protoplasmic and cell

wall constituents in the tumour. Apparently the tumour does not stimulate the normal tissue of the same beet root to convert sucrose into these substances itself, at least not to any marked extent.

TABLE V

COMPOSITION OF TUMOURS AND NORMAL TISSUE OF BEETS EXPRESSED IN TERMS OF THE CRUDE PROTEIN

Constituent	Healthy beets*	Normal tissue of tumorous beets*	Tumorous tissue*
Total ash	0.93	1.11	0.61
Free glucose	0.10	0.07	0.06
Free fructose	0.05	0.08	0.03
Sucrose	4.51	2.45	0.61
Starch	0.11	0.20	0.13
Ether extract	0.15	0.16	0.15
Pectins	0.93	0.66	0.66
Total uronic anhydride	1.20	0.93	0.64
Uronic anhydride (in holocellulose)	0.48	0.50	0.39
Pentosans (in holocellulose)	0.19	0.19	0.13
Cellulose	0.64	0.63	0.45
Acid lignin	0.38	0.40	0.30
Total methoxyl	0.078	0.076	0.063
Methoxyl insoluble in anhydrous ether	0.078	0.075	0.063
Methoxyl insoluble in anhydrous ether and hot ammonium oxalate (0.5%)	0.068	0.070	0.053

*These figures are the ratio: $\frac{\%\ \textit{substance (dry weight)}}{\%\ \textit{crude protein (dry weight)}}$

The results of Table V show that the ratio of cell wall materials (cellulose, lignin, methoxyl, pentosans, and uronic anhydride (in holocellulose)) to protoplasm is the same in the normal tissue of infected beets and the tissue of healthy beets but in the tumours there is a relatively greater concentration of protoplasm. This indicates the greater physiological activity to be expected in tumour cells and substantiates the well known concept that tumour cells are comparatively "young" cells. There is less than one-seventh the amount of sucrose per unit of protoplasm in tumours as in the healthy tissue of non-infected beets; the reducing sugars are also present in a lower amount per unit of protoplasm.

From Table II the ratio of cellulose to the other cell wall components can be calculated, and it will be observed that this does not vary much in the different tissues; this proves that no increase in cell wall incrustation takes place in tumour formation. The pectins show a peculiar behaviour. The ratios of cellulose to pectin in healthy beet tissue, normal tissue of infected beets, and tumorous tissue are respectively 0.68, 0.95, 0.68. Examination of the results in Table III indicates that pectins are apparently present in comparatively small amounts in the normal tissue of infected beets, thus indicating that the tumours have removed pectin from the normal tissue.

The values for total uronic anhydride reflect this change in pectin content. It is possible that it is necessary to distinguish between two different kinds of pectin:

(a) a reserve pectin,

(b) a structural pectin,

and to postulate the reserve pectin as that being transported to the tumours for transformation into other materials.

The results reported in this paper are in general agreement with those obtained by previous workers on this subject and the following observations seem warranted:

The crown gall organism exerts a local stimulus on the cells of the beet root causing them to proliferate, thus producing new cell wall and protoplasmic material. The latter is produced from sucrose supplied from the rest of the root; possibly pectins also are obtained similarly. The normal tissue of the tumorous root attempts to function normally and build up a store of sucrose. However considerable sucrose is lost to the tumour with the result that the plant as a whole probably manufactures much more sucrose than a non-infected plant since more dry matter is formed but, in the end, the infected plant contains less sucrose because it has been converted into other substances (protein, lipids, cell wall materials). This conversion of sucrose to protoplasmic and cell wall materials occurs in the tumorous tissue of the infected beet in which the normal tissue acts as a food reservoir. The normal tissue is apparently not stimulated by the bacteria to convert sucrose into other substances. Thus in the same beet root we have a tissue which is actively synthesizing new cell wall and protoplasm and a tissue of the same type that is relatively inactive, in this respect, and serves to store food.

Valuable information as to the nature of the mechanism of the conversion of sucrose into new living material might be obtained from a comparative study of the carbohydrate metabolism (respiration) of these tissues. It is quite possible that sugars are respired to give different intermediates in tissues that are synthesizing new material actively as compared with the intermediates formed in tissues whose chief function is to store sucrose, and beets infected with crown gall should provide suitable material for such a comparative study.*

Tentative Conclusions

Presentation of results of the type obtained in this investigation based exclusively on percentage of dry matter does not show the true effect of tumour formation on the physiological activity of beet root. It is necessary to calculate the results in such a way as to permit comparison of concentration of materials in the living tissue, the amount in an individual beet root, and the ratio of one substance to another, before any valid conclusions can be drawn regarding the effect of *P. tumefaciens*.

The crown gall organism stimulates growth of the root as a whole; more solid matter is produced in infected roots.

* *This is now in progress.*

The tumour contains cells that actively convert sucrose into new protoplasm and cell wall material. The cells of the tumour are richer in protoplasm than those of mature normal tissue.

The crown gall organism stimulates the tumour cells only, causing them to proliferate and synthesize living material from stored food.

The normal tissue of tumorous roots loses sucrose and possibly pectins to the tumours but apparently is not stimulated by the bacteria to synthesize protoplasmic and cell wall material. The increased dry weight of tumorous roots is due to synthesis that occurs in the tumours. The rest of the root is stunted. There is no increase in the degree of cell wall incrustation in tumours. The cell wall of the cells in tumours and normal tissue is similar in chemical composition. There is a greater concentration of cell wall material and protoplasm in tumours and a smaller concentration of sucrose.

An infected beet root contains less sucrose (although more solid matter) than a healthy beet root.

One of the major metabolic processes in tumours is the conversion of sucrose into protein.

The lignin in beets is probably of the same general nature as the lignin in woods, as shown in the ethanolysis results in the dried beets.

It is the authors' intention to carry out a study of the carbohydrate metabolism of these tumours to determine the manner in which it differs from that of normal tissue. It is possible that such a study may serve to indicate the relation between respiration and synthesis.

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